4-2016

Tolerance as a novel mechanism of Hessian fly control on wheat

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Graduate School Form
30 Updated 12/26/2015

PURDUE UNIVERSITY
GRADUATE SCHOOL
Thesis/Dissertation Acceptance

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By Kirsten E. Roe

Entitled

TOLERANCE AS A NOVEL MECHANISM OF HESSIAN FLY CONTROL ON WHEAT

For the degree of Master of Science in Entomology

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Approved by Major Professor(s): Dr. Brandon Schemerhorn

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Head of the Departmental Graduate Program Date

TOLERANCE AS A NOVEL MECHANISM OF HESSIAN FLY CONTROL ON WHEAT

A Thesis

Submitted to the Faculty

of

Purdue University

by

Kirsten E. Roe

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science in Entomology

May 2016

Purdue University

West Lafayette, Indiana

For my parents and friends who have supported me through my studies, research, and sickness. For my advisor who sacrificed time to help me. For my Savior who was my strength through every moment, even when my health tried to inhibit me.

ACKNOWLEDGEMENTS

I would like to thank Dr. Jeffrey Holland for help with statistical analysis and experimental design, and Dr. Hossam Moniem for assistance with statistics. We would like to thank Sue Cambron and Richard Smith for greenhouse assistance and Greg Marshall at Dupont Pioneer for providing seeds. The mention of any product does not constitute an endorsement by the USDA or ARS. This work was supported by USDA-ARS CRIS 3602-22000-1.

I am very grateful to Dr. Richard Shukle for all of his valuable advice, suggestions, and help as a member of my advisory committee and will miss his help and stories greatly.

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ABSTRACT

Roe, Kirsten E. Purdue University, May 2016. Tolerance as a Potential Method of Hessian Fly Control. Major Professor: Brandon Schemerhorn.

The effects of Hessian fly (*Mayetiola destructor* Say) infestation on the putative tolerant wheat line Pioneer® brand variety 25R78 were investigated at the seedling stage. Measurements, including leaf and tiller number, leaf growth rate, and total leaf lengths were recorded for two time intervals, 16 and 32 days post infestation (dpi). At 16 dpi, total leaf length changes and leaf growth rates were significantly lower for infested tolerant plants versus uninfested plants. No permanent growth effects occurred in the 32-day set. There were no significant differences in change in leaf length and leaf growth rate in infested tolerant plants compared to uninfested plants. Infested tolerant plants exhibited significantly higher leaf numbers per plant, but no significant difference in tiller numbers compared to uninfested plants. No dead red larvae were observed, differing from resistant plants commonly used and preventing selection pressure on fly populations.

Tolerant plants showed no significant effects on leaf or tiller number, total leaf length, or leaf growth rate due to infestation. The larvae observed on the tolerant plants were smaller compared to larvae on susceptible lines ‘Newton’ and Pioneer variety 25R75 with no significant difference from larvae on ‘Iris’ plants. Additionally, tolerant plants had significantly more visible larvae with greater distances of visible larvae from

the soil and from the first ligule and were the only plants with visible larvae above the first ligule. Tolerant plants showed no effect from infestation on head or tiller number, total seed number and weight, average seed number and weight, head height, head length, and other measurements. This was comparable to the infested resistant line, Pioneer® brand variety 25R32.

CHAPTER 1. REVIEW OF LITERATURE

1.1 Introduction

Hessian flies, or *Mayetiola destructor* (Say), are one of the most destructive insect pests of wheat (*Triticum aestivum*). Additionally, wheat is the most produced and consumed commercial crop globally (USDA, 2015), and because of this, controlling Hessian flies to reduce yield loss is essential. Currently, one of the primary control methods being researched and used is the wheat resistance gene, or *R* gene, encoding resistance to Hessian flies.

However, because of selection pressures placed on fly populations due to larval antibiosis, new biotypes can evolve to be able to overcome plant resistance rapidly (Ratcliffe and Hatchett, 1997). Consequently, other control methods need to be researched to use alongside resistance genes. One of these methods is the use of wheat tolerance to Hessian flies. In contrast with resistance, tolerance might not exert selective pressure on the fly population, preventing the development of resistant fly biotypes.

This study investigated the potential of putative tolerant Pioneer brand variety 25R78 as a method of Hessian fly control. This included analyzing growth and yield effects on the tolerant line by Hessian fly infestation as well as the effect of the tolerant

line on the Hessian fly larvae. If the putative tolerant plants could survive larval feeding with the absence of a negative effect on plant growth, this could translate into prevention of yield loss. In order to determine the degree of tolerance in Pioneer 25R78, several experiments were performed. The first experiment's purpose was to determine infestation growth effects on young plants at two time intervals: 16 and 32 days post infestation (dpi). To confirm these results, the second experiment combined analyzing larval effects on tolerant plants and plant effects on the larvae at 20 dpi. The last experiment was performed to analyze the yield effects of infestation on tolerant wheat since yield would be the most important aspect for farmers.

This project provides data on the potential efficacy of tolerance as a tool for managing Hessian fly infestations, including tolerant plants' ability to survive infestation without killing Hessian fly larvae or putting selection pressures on fly populations. The data from this study may help reduce selection pressures in fly populations, improve resistance gene efficacy, and reduce yield loss.

1.2 Wheat

1.2.1 Wheat Production

Bread wheat (*Triticum aestivum* L.) is the most widely produced and consumed cultivated crop globally (Fisher, 2009). Wheat is the largest source of food calories grown on the largest crop area, making up the most traded crop globally with approximately 124 million metric tons (MMT) produced in the United States alone in 2009 (Fisher, 2009). In 2008–2009, 680 MMT were cultivated on 225 million hectares

globally (Fisher, 2009). According to a report from the USDA, global wheat production in the 2014–2015 fiscal year had an estimated output of 725.34 million metric tons (MMT). Rice only had a total output of 478.19 MMT, while coarse grains, including corn, rye, millet, and sorghum, had a combined production output of 1296.97 MMT (USDA, 2015). United States production showed a total output of 55.84 MMT and total consumption of 31.53 MMT. Consequently, wheat is vital to the global community as a source of food and income.

1.2.2 Wheat Classifications

Wheat in the US is classified as either hard red winter (HRW), soft red winter (SRW), soft white (SW), hard white (HW), durum, or hard red spring wheat (HRSW) (Briggle and Reitz, 1963; Maghirang et al., 2006). The red or white classification depends on the corresponding grain color, while grain hardness determines whether the wheat is considered hard or soft (Taylor, 1921). Winter or spring wheat is classified by the corresponding growing season for that variety. Winter wheat is planted and germinates in the fall, while the vegetative phase occurs in winter. A period of time in cold temperatures (0–5°C) is necessary for the plant to head in the spring and grow to maturation. On the other hand, spring wheat is generally planted in the spring. In countries with mild winters, spring wheat can be planted in fall (Curtis, 2002).

Generally, HRW and HRS wheat are chosen for breadmaking due to multiple characteristics such as seed protein content and hardness, as well as seed size, average gluten index, and loaf volume potential (Finney, 1965; Maghirang et al., 2006). However, research shows that HRS wheat has higher flour and grain quality than HRW in areas

including geometric mean, diameter, dough weight, crumb grain score, grain protein content, milling, flour, and dough measurements, and baking properties (Maghirang et al., 2006). Winter wheat has a greater resistance to drought and can also mature earlier in the season than spring wheat (Taylor, 1921). Soft wheat is typically used for cakes, pastries, and cookies. Durum wheat, *Triticum durum*, is generally used as semolina, a key ingredient for pasta. Durum wheat can also be used to make couscous and medium-dense bread (Pena, 2002).

1.2.3 Wheat Life Stages

Bread wheat has three main phases: vegetative, reproductive, and grain filling (Kirby and Appleyard, 1987; Guo et al., 2015). The vegetative phase includes leaf initiation. The reproductive phase includes spikelet initiation, floret initiation, active spike growth, active stem growth, and floret death. The grain filling phase includes grain set and grain filling. Anthesis, or flowering, requires several conditions to be met. These steps make up the pre-anthesis phase. The first step is leaf development. Collar initiation occurs and the plant continues into the second step of spikelet development. The second and third step involves terminal spikelet formation. The third step of the pre-anthesis phase is floret development which consists of floret and primordia formation and anthesis.

1.3 Hessian Fly

1.3.1 Hessian Fly Impact

Hessian flies, or *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), are one of the most detrimental insect pests of wheat in primary cereal-growing regions such as

Southern Europe, North America, and North Africa (El Bouhssini, 1996; Harris et al., 2003). Hessian flies are believed to have originated in the Fertile Crescent, spreading through Europe, Siberia, central Asia, and the Mediterranean region (Barnes, 1956). They were first reported in North America in 1777 in New York. Now, they range throughout each wheat-growing region (Barnes, 1956).

Hessian flies are members of the gall midge family. Gall-forming insects typically induce galls such as the formation of abnormal plant organs, tissues, or cells (Harris et al., 2003). Instead of the normal gall, the virulent Hessian fly larva forms gall-like nutritive tissue, known as the feeding site, by inducing new, unexpanded leaves to develop.

One report estimated Hessian fly damage of wheat in the United States could reach up to $100 million per year (Cartwright and Jones, 1953). In 1989, Georgia alone experienced losses of $28 million (Hudson et al. 1991). In 2004, a study in Oregon showed a 66 and 68% increase in grain yield for resistant genotypes over susceptible genotypes (Smiley et al., 2004). The susceptible genotypes produced two–thirds less grain than the resistant genotypes at intermediate infestation (50% or fewer infested plants) with a reduction in grain quality (Smiley et al., 2004). One major issue with Hessian fly infestations is the ability to have multiple generations in one growing season, infesting both winter and spring wheat (Porter et al., 2009).

Severe crop loss due to Hessian fly infestations has been noted worldwide. For example, in Morocco, Hessian fly damage can lead to a complete crop loss, when infestations in the fall are high and occur during the more vulnerable younger crop stages such as the seedling stage (El Bouhssini, 1996). Lhaloui et al. (1992a) used the

insecticide Furadan 5G to control Hessian flies in Morocco, with infestations still causing an estimated loss of 42%. Another study, on the other hand, used near susceptible and resistant isogenic lines of bread wheat (*Triticum aestivum* L.) and resulted in a 36% yield loss (Amri et al., 1992). Durum wheat (*T. turgidum* L. var. *durum*) was also studied, demonstrating a 32% yield loss due to Hessian fly infestation (Lhaloui et al., 1992b).

1.3.2 Hessian Fly Life Cycle

Hessian flies can successfully attack bread and durum wheat (Lhaloui et al., 1996; Ratcliff and Hatchett, 1997), as well as other grasses such as barley, triticale, rye, and wild grasses (Harris et al., 2001; Harris, 2003). Hessian flies can have 1–6 generations a year depending on the climate and latitude (Buntin and Chapin, 1990; Wellso, 1991). In the spring, 1–3 generations can develop before aestivation, while in the fall, 1–3 generations can occur before diapause (Buntin and Chapin, 1990; Wellso, 1991).

Multiple authors have detailed the Hessian fly life cycle (Painter, 1951; Ratcliffe and Hatchett, 1997; Royer and Giles, 2009). The Hessian fly's life cycle lasts 20–61 days (Painter, 1951) and begins when an adult female mates and lays 250–300 eggs during her 3–4-day lifespan. The eggs are reddish-orange and are laid on the adaxial (upper) side of leaves. After 3–10 days, 1st-instars eclose and crawl down the plant to a leaf base under the leaf sheath, generally between the first and second leaves, where it attacks the outer surface of the leaf sheath of the youngest leaves close to the meristematic crown (McColloch and Yuasa, 1917; Painter, 1951; Refai et al., 1955). This migration takes 4–12 hours (McColloch and Yuasa, 1917). The larvae then establish a feeding site after 2–3 days where they continue to feed for the rest of that larval stage and through the second

instar, causing leaf growth deficits in the seedling (Harris et al., 2006). Feeding at this site lasts for 10–14 days during the first and second instar larvae with the largest consumption on the fifth day. The first stadium lasts six days while the second stadium lasts five to six days (Gallun and Langston, 1963; Gagne and Hatchett, 1989).

Unlike the first instar, the creeping pads are absent from the second instar, prohibiting larval movement after the first instar (Harris et al., 2006). If the feeding site is not effective or fails to form, the larvae cannot move to another location after the first instar. The third instar does not feed and remains enclosed in a puparium (the skin of the second instar). The larva will diapause during the winter or summer. Under appropriate conditions, the larva becomes a pupa, then an adult, within the puparium. This development takes 6–7 days after which the adult fly ecloses (Sosa and Gallun, 1973).

1.3.3 *R-Avr* Interactions

Research on the control of the Hessian fly on wheat has been primarily focused on host *R* genes, or resistance genes. These *R* genes are also referred to as *H* genes when part of the wheat-Hessian fly interaction. Currently, 35 resistance genes have been identified: *H1–H34* and *Hdic* (Sardesai et al., 2005; Li et al., 2013; McDonald et al., 2014). These genes act as a gene-for-gene interaction between the plant *Resistance* (*R*) gene and a Hessian fly *Avirulence* (*AVR*) gene (Hatchett and Gallun, 1970; Gallun, 1977; Stuart et al., 2008). This interaction was hypothesized by Flor (1955) for flax-rust interactions. Flor predicted that each plant *R* gene has a corresponding parasite *AVR* gene. Also, *R/AVR* interactions have been discovered in other plant-pathogen interactions such as

microbes, fungi, insects, and nematodes (Subramanyam et al., 2005; Bent and Mackey, 2007).

Avirulence genes encode for proteins that can act as both “elicitors” and “effectors.” The “elicitor” function triggers the plant’s defense system through recognition in a dominant, incompatible gene-for-gene interaction. It is unknown currently whether “elicitors’ trigger recognition through direct or indirect molecular interactions (Nimchuk et al., 2003). When avirulent larvae secrete effectors into a resistant plant, the plant recognizes one or more effectors and a defense response, called effector-triggered immunity, occurs, triggering an up-regulation of the gene encoding *Hfr-1* (Williams et al., 2002). This protein is a plant lectin that might affect the avirulent larva’s ability to feed by preventing the establishment of a successful feeding site and causing the larvae to experience writhing and increased searching time (Williams et al., 2008).

The “effector” function benefits the parasite’s colonization of the plant. In the case of HF and wheat, effectors act in several ways. First, they reprogram plant cells to amplify nutrient production including proteins (Shukle et al., 1992), sugars (Refai et al., 1955), and free amino acids (Saltzmann et al., 2008). The nutritive tissue is formed when effectors lead to surface wax composition changes and changes in cutin concentrations. A decrease in cutin monomers and cutin coverage, as well as a small increase in wax coverage, occurs in compatible interactions (Kosma et al., 2010). Induced epidermal permeability modulates resistance and susceptibility of wheat seedlings to herbivory by Hessian fly larvae.

R genes fail when the HF loses an *Avirulence* gene through mutations that either decreases protein production or change the amino acid sequence, leading to the loss of the effector (Gallun, 1977; Rider et al., 2002; Harris et al., 2006; Stuart et al. 2008). This could affect the larva's ability to colonize as efficiently, but prevents early recognition by the plant and reduces or prevents the induction of plant defenses. In the case of *H13*, insertions in one HF gene allows the larva to escape *H13*-directed effector-triggered immunity by encoding a similar protein to an effector. This mutated gene is an *Avr* gene found in virulent Hessian flies and is called *vH13* (Aggarwal et al., 2014).

Larvae pierce the leaf surface with minute mandibles to secrete saliva with effectors produced in salivary glands (Hatchett et al., 1990). These mandibles and the punctures they make are only several micrometers long and less than 0.1μm in diameter (Hatchett et al., 1990; Harris et al., 2006). Epidermal permeability occurs at the feeding site and differs between compatible and incompatible interactions. In both cases, the larvae may successfully secrete salivary effectors into the plant cells, due to the epidermal permeability (Williams et al., 2011). However, resistant plants will have temporary, localized permeability close to the feeding site with no down-regulation of genes encoding proteins that benefit surface structure (Kosma et al, 2010). In one study, GDSL-motif lipase/hydrolase mRNA in infested resistant wheat increased 51-fold in number (Williams et al., 2011). This mRNA may affect cuticle reorganization and may increase epidermal permeability (Yeats et al., 2010). However, the transcripts rapidly decrease in abundance, allowing permeability to return to pre-infestation levels (Williams et al., 2011). The temporary permeability allows for defense molecules, such as lectins

and reactive oxygen species, to be delivered to the site of larval attack (Giovanini et al., 2007; Subramanyam et al., 2008; Liu et al., 2010; Williams et al., 2011).

Additionally, in incompatible interactions, only a few local vascular bundles and epidermal cells showed permeability, and the levels of staining demonstrating permeability returned to near-control amounts by four days after hatching (Williams et al., 2011). This permeability might play a role in allowing defense molecule movement to the feeding site before the site is repaired. Only one virulent larva induced permeability approximately 3.6 mm both posteriorly and anteriorly from the feeding site (Williams et al., 2011). The greater the number of larvae, the larger the permeability becomes in a susceptible plant.

1.3.4 Compatible interactions

Compatible interactions between the Hessian fly and wheat occur when the virulent larva creates an effective feeding site. In compatible interactions at the seedling stage (fall infestation), the Hessian fly larva can stunt the plant irreversibly, making the plant susceptible to winter kill. In addition, the plant's leaves will become dark green and heavy infestations can kill the plant (Byers and Gallun, 1972). During the spring infestation when wheat is at the jointing stage, larvae can reduce or prevent normal stem elongation while preventing nutrients from reaching the developing head of grain (Buntin, 1999). At the feeding site, genes that encode for amino acids are up-regulated in compatible interactions. Two to three days after the initial attack, nutritive cells begin to form in the epidermal and mesophyll cells at the base of the third leaf. These nutritive cells showed accumulation in multiple organelles such as mitochondria, Golgi, rough

endoplasmic reticulum, and proplastids. The nucleus shrinks and becomes misshapen (Harris et al., 2006). Shortly after nutritive cell formation begins, the cells begin to breakdown. The breakdown includes invagination of the nuclei and cytoplasmic degradation. Thinning of epidermal cell walls leads to further breakdown and cell rupture (Harris et al., 2006).

As larvae become sessile 2nd-instars, amino acids accumulate in the plant near the feeding site. The main free amino acids accumulated include tyrosine, phenylalanine, alanine, aspartate, histinine, methionine, glutamate, glycine, and serine (Saltzmann et al., 2008). The greatest amount of accumulation occurs for tyrosine and phenylalanine, with the addition of mRNAs for enzymes that synthesize these two amino acids (Saltzmann et al., 2008). Phenylalanine acts as a precursor to tyrosine and tyrosine acts a precursor for quinone and polyphenol production. These products help melanize the insect cuticle at pupation (Heady et al., 1982; Dindo et al., 2006). The newly accumulated organelles and amino acids are released and made accessible to the larva once the cell walls thin and rupture (Harris et al., 2006; Saltzmann et al., 2008).

1.3.5 Incompatible Interactions

Incompatible interactions occur when an avirulent larva does not succeed in establishing a feeding site and dies on a resistant plant (Ratcliffe and Hatchett, 1997; Agrios, 1997; Harris et al., 2006). This incompatibility occurs due to the dominant or partially dominant alleles of major resistance loci, *H1-H32*. In incompatible interactions, the avirulent larvae die after 3–5 days at the feeding site without any growth (Shukle et al., 1990).

Hessian fly larvae successfully pierce the outer cell wall of epidermal cells in resistant plants, however, these plants have smaller ruptures in the outer cell walls than susceptible plants (Harris et al., 2010; Rohfritsch, 1992). Immediately after the larval attack, localized epidermal cells die and reactive oxygen species accumulate at the feeding site (Grover, 1995, Harris et al., 2010, Liu et al., 2010). Adjacent epidermal cells survive and undergo changes as a method of induced resistance to potentially resist penetration and reduce food access to larvae (Hardham et al., 2007; Harris et al., 2010). The surrounding epidermal and mesophyll cells exhibit enlarged mitochondria, intricate Golgi complex-endoplasmic reticulum with ribosomes, reinforced cell walls, separation of the plasma membrane and cell wall, and an increase in plasma membrane surface area (Harris et al., 2010).

Resistant plants (*H6*, *H9*, and *H13*) show the initial effects of larval attack on seedling epidermal cells through the growth deficits of leaves (Anderson and Harris, 2008). Resistant plants show a smaller loss in growth than susceptible plants in the third leaf after 36–60 hours and 60–84 hours after larval attack. The fourth leaf also has growth loss beginning at 156–160 hours after infestation until growth is completed. This effect does not begin until the larvae have died. These delayed effects might be due to costs incurred by induced defenses of the third leaf (Heil, 2002). Another possibility could be the effect of larval virulence products such as effectors. Although the feeding site fails to be produced, the effectors or other compounds could affect production or movement of photoassimilates, affecting resource allocation and photosynthesis. (Weis et al., 1988; Fay et al., 1993; Jankiewicz et al., 1970; Anderson and Harris, 2008). Some of these

deficits in *H6* occurred earlier than susceptible plants, possibly supporting the model of *R–Avr* interactions (Nimchuk et al., 2003).

The disadvantage of resistance genes is that the selection pressure exerted on surviving flies leads to the increased frequency of new virulent biotypes capable of overcoming *R* genes. An increased frequency of the virulent biotypes can occur any time after *R* gene deployment, sometimes occurring immediately after the release of the line or within 5–10 years (Ratcliffe and Hatchett, 1997; Ratcliffe et al., 2000; Cambron et al., 2010). For example, in the southeastern United States, biotypes exist that are virulent on wheat with *R* genes *H3, H5, H6,* and *H7H8* (Cambron et al., 2010). In that region, only six *R* genes (*H12, H18, H24, H25, H26,* and *H33*) are predicted to be effective against the fly populations (Cambron et al., 2010). Because of this constant race against Hessian fly virulence, various methods must be used to control Hessian fly. This includes releasing new *R* genes, planting after the "fly-free" date, and using insecticide-soaked seeds. However, these methods may not be the only control options; another potential method of Hessian fly control involves wheat tolerance.

1.4 Tolerance

1.4.1 Definition of Tolerance versus Resistance

One definition of tolerance is the ability of the plant to continue growing despite insect attack (Reese et al., 1994). A second definition builds on the first by defining tolerance in plants as the ability of the plant to recover in growth as well as potentially

reproduce (Strauss and Agrawal, 1999). The third definition incorporates tolerance as a component of plant resistance.

Plant resistance refers to any plant trait that can decrease the performance or preference of a herbivore (Rosenthal and Kotanen, 1994). This resistance may take two forms. The first is to avoid damage through escape (antixenosis) or defense (antibiosis). Antixenosis includes any method that affects the insect's ability to find, attack, feed on, or to colonize a plant, while antibiosis refers to methods that harm, kill, or affect growth of the feeding stages of a pest (Painter, 1951). The second form of resistance is tolerance of damage according to Painter (1951). Tolerance, in this case, is the plant's ability to maintain its fitness after herbivore damage through reproduction and growth (Rosenthal and Kotanen, 1994). Tolerance may also include compensation, or allowing regrowth, compared to a susceptible host with a similar population of pests (Painter, 1951). This third definition takes into account the role tolerance plays in plant resistance.

However, resistance in wheat to Hessian fly attack differs from general plant resistance and occurs through the *R-Avr* interaction. This resistance causes larval death through its own form of antibiosis 3–5 days after insect attack without allowing growth and does not consist of antixenosis (Shukle et al., 1990). Unlike normal herbivore feeding where tissue damage occurs, Hessian fly larvae feed by altering the development and nutrient content of the epidermal cells under the leaf sheath. The damage does not occur directly to the leaves, but occurs within the leaf sheath of the plant where induced epidermal cells become permeable and rupture to form nutritive tissue (Shukle et al., 1992; Williams et al., 2011).

Each of the definitions of tolerance builds on the basic premise that tolerant plants can grow or suffer minimal yield impacts from insect attack without leading to a buildup of pest resistance. Tolerance is dependent on both the insect and the plant. Tolerance relates to an insect's effect on a plant where a greater tolerance level results in less damage to the plant. Tolerance also relates to the plant's response to insect injury through recovery and growth (Reese et al., 1994). For this study, the first two definitions will be the main references for tolerance in wheat.

1.4.2 Factors Influencing Tolerance

The regrowth in tolerant plants may be dependent on physiological features such as the intrinsic growth rate of the plant (Herms and Mattson, 1992). A slower growth rate might affect how well a plant regrows or replaces damaged tissue (Coley et al., 1985). Other intrinsic physiological factors contributing to initial regrowth in *Gramineae* species include stored reserves of carbon as well as compensatory photosynthesis and increased nutrient uptake (Richards, 1993; Welter, 1989). An increased rate of compensatory photosynthesis occurs after damage while the carbon reserves in the roots allow for carbon allocation for reproduction (Danckwerts, 1993; Briske et al., 1996).

The last primary intrinsic factors include the ability to mobilize root carbon stores to plant shoots after damage occurs, as well as to show an increase in tillering or branching (Mabry and Wayne, 1997; Houle and Simard, 1996; Rosenthal and Welter, 1995; Mutkainen et al., 1994; Briske et al., 1996). Morphological features also support tolerance such as protected meristems (Coughenour, 1985). Another factor, the timing of damage, contributes to tolerance. For example, young seedlings can be less tolerant than

mature plants and the flowering stage is the most sensitive to damage (Crawley, 1989; Trumble et al., 1993).

Several external factors affecting tolerance include abiotic factors. Scarce abiotic factors, including light, water, and nutrient availability, might decrease tolerance (Chapin III and McNaughton, 1989). Competition can also reduce tolerance by affecting the availability of these resources (Dirzo, 1984). Another important factor includes the type of herbivore and their type of damage. Damage to stems or roots as well as sap feeding can inhibit tolerance more than leaf damage or removal (Karban and Strauss, 1993; Meyer, 1993). Damage or loss of vascular tissue or meristems directly affects recovery and regrowth compared to a loss of photosynthetic leaf material.

1.4.3 Advantages and Disadvantages of Tolerance

One potential benefit of tolerance in any crop includes the plant's ability to support and maintain natural enemy populations by not decreasing prey numbers through antixenosis (non-preference) or antibiosis (Horber, 1972). Tolerance could also increase economic injury levels, known as the smallest number of pests to cause economic damage (Stern et al., 1959; Pedigo et al., 1986). This might reduce or delay required insecticide use (Reese et al., 1994). Thirdly, tolerance does not impose selection pressures that lead to the formation of novel biotypes (Reese et al., 1994; Strauss and Agrawal, 1999). The lack of selection pressure is due to the fact that tolerance is a plant reaction to insect attack with no direct effect on insect physiology, growth, reproduction, biology, or fitness (Reese et al., 1994). This might make tolerance more evolutionarily stable than defense such as antibiosis (Gould, 1983; Kennedy et al., 1987).

The primary disadvantage with tolerance is the difficulty in separating it from antixenosis or antibiosis as well as quantifying or measuring it (Reese et al., 1994). It is difficult to measure or quantify tolerance in plants because it is the plant that requires measurement because tolerance is defined under plant production. For different species of aphids, there have been many attempts to measure tolerance including measuring damage, seedling survival, and height on wheat (Starks and Mirkes, 1979; Wood, 1961).

1.5 Tolerance in Wheat

Very little research has focused on tolerance in wheat to Hessian flies and there is scant research on partial tolerance. Marquillo hybrids, a cross of *R* gene *H18*-containing Marquillo and winter wheat, were found to be able to withstand Hessian fly infestation by surviving and providing yield even under heavy infestation (Agricultural Experiment Station Kansas State, 1940). This tolerance, when combined with poor larval survival, was a potent resistance trait. The wheat line ‘Superb’ can reduce yield loss from fly infestations by up to 65% compared to susceptible lines such as ‘AC Barrie’ due to the partial tolerance and antibiosis present in the line (Wise et al., 2006). These researchers considered tolerance as the ability of wheat stems to survive larval feeding without snapping (Wise et al., 2006).

‘Superb’ showed partial larval death which could place selection pressure on fly populations. Pioneer variety 25R78 was screened by Sue Cambron in the USDA-ARS greenhouse in West Lafayette, IN. The screening showed potential tolerance in that flies continued to emerge from the plants after infestation, but plants did not die or stunt as dramatically. For this reason, Pioneer variety 25R78 was chosen as the putative tolerant

line to be studied in this experiment. The susceptible Pioneer variety 25R75 was chosen to act as a control for this tolerant line.

It is theorized that tolerance in wheat might be used alongside resistance to help offset allocation expenses for *R* genes such as reduced yield, seed protein, and seed weight (Smedegaard-Petersen and Stølen, 1981; Anderson et al., 2011). Initially, there might be an allocation cost for a resistant and tolerant plant, but the cost would eventually disappear due to growth compensation (Anderson et al., 2011). This compensation could be completed via carbon deployment to larvae-inaccessible regions or redistribution of the carbon for growth after the larvae die (Schwachtje et al., 2006).

There are several reasons why tolerance to Hessian flies is believed to be present in wheat. First, susceptible 'Newton' has the ability to trigger growth through tiller production from an axillary coleoptile meristem in order to survive infestation (Anderson and Harris, 2006). Second, some infested resistant lines have the ability to reflect compensation in growth through the production of superior qualities compared to uninfested plants when infested such as greater seed numbers and heads as well as taller plants. These resistant lines also show superior qualities when infested compared to uninfested. For example, infested *H6* plants were taller, *H13* plants have a greater number of seeds and greater total seed weights, and *H9* and *H13* plants show more seed heads compared to susceptible lines (Anderson et al., 2011).

However, Hessian fly tolerance might not only be present in resistant and susceptible wheat lines, but could be a unique plant defense response by a wheat line. We asked whether or not the putative tolerant Pioneer® brand variety Pioneer variety 25R78 could survive Hessian fly infestation, continue to grow, and not directly kill the larvae via

antibiosis. We asked if leaf growth rates, leaf and tiller numbers, and leaf lengths would be able to recover from initial stunting. This would make the tolerant wheat line distinct from susceptible lines due to the lack of main tiller leaf growth in susceptible lines.

In an experiment done by Anderson and Harris (2006), infested susceptible line 'Newton' demonstrated poor main tiller (stem) leaf growth with large growth deficits (shorter leaves) in all leaves as well as significant growth deficits in each individual leaf compared to uninfested plants. This growth was measured by measuring the length of each leaf from the lamina tip to either the ligule or to the leaf base (if the ligule was not yet formed and visible). Growth deficits would show smaller leaf lengths in either total leaf length or individual leaf lengths.

In the incompatible interactions, each leaf soon after infestation showed growth effects for the region of greatest growth, evident in shorter third and fourth leaves. However, the fifth and main tiller leaves showed no growth deficits because their peak growth occurred after larvae had been killed in the *R* gene interaction (Anderson and Harris, 2006). Despite the initial growth deficits, total leaf lengths were similar to or greater than those of uninfested plants, demonstrating that resistance can prevent serious or permanent growth deficits (Anderson and Harris, 2006). Normal growth or recovery of leaves of the main stem would signal a difference between the tolerant line Pioneer variety 25R78 and the susceptible line 'Newton'. No larval death would signify a difference between Pioneer variety 25R78 and resistant lines.

Tolerance has the potential to be an effective control method for Hessian fly as preliminary studies show that it does not entail antibiosis of the Hessian fly larvae through death, but might still allow the plant to produce yield that is not significantly

different from that of resistant lines. This could reduce or prevent selection pressure exertion, reducing or preventing new biotype formation in fly populations.

1.6 Research Objectives

The overall purpose of the experiments conducted for this thesis was to discern the ability of putative tolerant Pioneer variety 25R78 to survive despite Hessian fly infestation without killing larvae and to determine the efficacy of this line as a method of Hessian fly control. If the fly populations are not reduced by fatal larval antibiosis, a tolerant line could be used as a refuge crop alongside resistant varieties in the same field, increasing the longevity of the resistance genes.

1.6.1 Specific Objectives

Consequently, the three goals of this study stated in Chapter 1 include:

1. Investigate the growth effects from Hessian fly infestation on putative tolerant Pioneer variety 25R78 in two time sets: 16 and 32 days post infestation (dpi).
2. Further investigate the mechanism of tolerance through improved study design with the following objectives:

    A) Investigate the growth effects of Hessian fly infestation on putative tolerant Pioneer variety 25R78 at 20 dpi.

    B) Investigate the effects of tolerant plants on Hessian fly larvae, including size, survival, and position on the plant.

3. Investigate the effects of Hessian fly infestation on the yield of tolerant plants.

The overall hypothesis is that Pioneer variety 25R78 displays tolerance. It was predicted that Pioneer variety 25R78 would stunt and recover in growth, demonstrating no permanent growth effects and growth comparable to uninfested controls. The absence of permanent growth effects would be accompanied by successful larvae survival and the presence of larvae outside the leaf sheath.

1.7 References

Aggarwal, R., S. Subramanyam, C. Zhao, M.S. Chen, M.O. Harris, J.J. Stuart. 2014. Avirulence effector discovery in a plant galling and plant parasitic arthropod, the Hessian fly (*Mayetiola destructor*). PLOSone. 9(6): 1–8.

Agricultural Experiment Station Kansas State College of Agriculture and Applied Science. 1940. Transference of Hessian Fly Resistance and Other Characteristics of Marquillo Spring Wheat to Winter Wheat. Technical Bulletin No. 49. Kansas State Printing Plant. Topeka, KS.

Agrios, G. N. 1997. Plant diseases caused by Mollicutes: Phytoplasmas and spiroplasmas, pp 457–470. In: Agrios, G.N. (Ed.), Plant Pathology, Academic Press, New York.

Amri, A, M. El Bouhssini, S. Lhaloui, T.S. Cox, J.H. Hatchett. 1992. Estimates of yield loss due to Hessian fly (Diptera: Cecidomyiidae) on bread wheat using near-isogenic lines. Al Awamia 77: 75–87.

Anderson, K.G. M.O. Harris. 2006. Does *R* gene resistance allow wheat to prevent plant growth effects associated with Hessian fly (Diptera: Cecidomyiidae) attack? Plant Resistance. 99(5): 1842–1853.

Anderson, K.G., M.O. Harris. 2008. Leaf growth signals the onset of effective resistance against Hessian fly larvae. Entomol. Exp. Appl. 128(1): 184–195.

Anderson, K.M, Q. Kang, J. Reber, M.O. Harris. 2011. No fitness cost for wheat's *H* gene-mediated resistance to Hessian Fly (Diptera: Cecidomyiidae). J. Econ. Entom. 104(4): 1393–1405.

Barnes, H.F. 1956. Gall midges of cereal crops, vol. 7. Crosby Lockwood and Sons, London, United Kingdom.

Benbi, D.K. 1994. Prediction of leaf area indices and yields of wheat. 122: 13–20.

Bent, A.F., D. Mackey. 2007. Elicitors, effectors, and *R* genes: the new paradigm and a lifetime supply of questions. Ann. Rev. Phytopathol. 45: 399–436.

Berzonsky, W.A., H. Ding, S.D. Haley, M.O. Harris, R.J. Lamb, R.I.H. McKenzie, H.W. Ohm, F.L. Patterson, F.B. Peairs, D.R. Porter. 2003. Breeding wheat for resistance to insects. Plant Breeding. 22: 221–297.

Briggle, L.W., L.P. Reitz. 1963. Classification of Triticum Species and of Wheat Varieties Grown in the United States. Technical Bulletin 1278. Washington. United States Department of Agriculture.

Briske, D.D., T.W. Boutton, Z. Wang. 1996. Contribution of flexible allocation priorities to herbivory tolerance in C-4 perennial grasses: an evaluation with ^{13}C labeling. Oecologia. 105: 151–159.

Buntin, G., J.W. Chapin. 1990. Biology of Hessian fly (Diptera: Cecidomyiidae) in the southeastern United States: geographic variation and temperature-dependent phenology. J. Econom. Entomol. 83: 1015–1024.

Buntin, G. D. 1999. Hessian Fly (Diptera: Cecidomyiidae) injury and loss of winter wheat grain yield and quality. J. Econ. Entomol. 92: 1190–1197.

Byers, R.A., R.L. Gallun. 1972. Ability of the Hessian fly to stunt winter wheat. 1. Effects of larval feeding on elongation of leaves. J. Econ. Entomol. 65: 955–958.

Cambron, S.E., G.D. Buntin, R. Weisz, J.D. Holland, K.L. Flanders, B.J. Schemerhorn, R.H. Shukle. 2010. Virulence in Hessian fly (Diptera: Cecidomyiidae) field collections from the southeastern United States to 21 resistance genes in wheat. J. Econ. Entomol. 103: 2229–2235.

Cartwright, W.B. and E.T. Jones. 1953. The Hessian fly and how losses from it can be avoided. USDA Farmers' Bul. 162: 1–9.

Chapin III, F.S., S.J. McNaughton. 1989. Lack of compensatory growth under phosphorous deficiency in grazing-adapted grasses from the Serengeti Plains. Oecologia. 79: 551–557.

Chowdhry, M.A., M. Ali, G.M. Subhani, I. Khaliq. 2000. Path coefficient analysis for water use efficiency, evapo-transpiration efficiency, transpiration efficiency and some yield related traits in wheat. Pak. J. Biol. Sci. 3: 313–317.

Coley, P.D., J.P. Bryant, F.S. III Chapin. 1985. Resource availability and plant antiherbivore defense. Science. 230: 895–899.

Coughenour, M.B. 1985. Graminoid responses to grazing by large herbivores: adaptations, exaptations, and interacting processes. Ann. Miss. Bot. Gard. 72(4): 852–863.

Crawley, M.J. 2007. Mixed Effects Models, pp. 630–637. *In* M.J. Crawley (ed.), The R Book. John Wiley & Sons, Ltd., West Sussex, United Kingdom.

Curtis, B. 2002. Wheat in the World. Bread Wheat: Improvement and Production. Rome: Food and Agriculture Organization of the United Nations.

Danckwerts, J.E. 1993. Reserve carbon and photosynthesis: their role in regrowth of Themeda triandra, a widely distributed subtropical graminaceous species. Funct. Ecol. 7: 634–641.

Dindo, M.L., S. Grenier, L. Sighinolfi, P. Baronio. 2006. Biological and biochemical differences between in vitro- and in vivo-reared *Exorista larvarum*. Entomol. Exp. Appl. 120: 167–174.

Dirzo, R. 1984. Herbivory: a phytocentric perspective, pp 141–165. *In*: Dirzo R., J. Saruhkan (Eds.), Perspectives on Plant Population Ecology, Sinauer.

El Bouhssini, M., S. Lhaloui, A. Amri, M. Jlibene, J.H. Hatchett, N. Nssarellah, M. Nachitt. 1996. Wheat genetic control of Hessian fly (Diptera: Cecidomyiidae) in Morocco. Field Crops Res. 45: 111–114.

Fay, P.A., D.C. Hartnett, A.K. Knapp. 1993. Increased photosynthesis and water potentials in *Silphium integrifolium* galled by cynipid wasps. Oeceologia. 93: 114–120.

Finney, K.F. 1965. Evaluation of wheat quality, pp 73–82. *In*: Food Quality: Effects of Production Practices and Processing, Publication No. 77, AAAS, Washington, DC.

Fisher, R.A. 2009. Foreword, p. xvii. *In*: B.F. Carver (Ed.), Wheat science and trade, Wiley-Blackwell, Ames, IA.

Flor, H.H. 1955. Host-parasite interaction in flax rust-its genetics and other implications. Phytopathology 45: 680–685.

Foster, J.E., J.E. Araya, G.G. Safranski, S.E. Cambron, P.L. Taylor. 1988. Rearing Hessian fly for use in the development of resistant wheat. Purdue Univ. Argic. Exp. Stn. Bull. 536: 1–16.

Gagne, R., J.H. Hatchett. 1989. Instars of the Hessian fly. Ann. Entomol. Soc. Am. 82: 73–79.

Gallun, R.L., R. Langston. 1963. Feeding habits of Hessian fly larvae on ^{32}P-labelled resistant and susceptible wheat seedlings. J. Econ. Entomol. 56: 702–706.

Gallun, R.L. 1977. Genetic basis of Hessian fly epidemics. Ann. N.Y. Acad. Sci. 287: 222–229.

Gertz, A.K. and C.E. Bach. 1995. Effects of light and nutrients on tomato plant compensation for herbivory by Manduca sexta (Lepidoptera: Sphingidae). Great Lakes Entomol. 27: 217–222.

Giovanini, M.P., K.D. Saltzmann, D.P. Puthoff, M. Gonzalo, H.W. Ohm, C.E. Williams. 2007. A novel wheat gene encoding a putative chitin-binding lectin is associated with resistance against Hessian fly. Mol. Plant Path. 8: 69–82.

Grover, P.B. 1995. Hypersensitive response of wheat to the Hessian fly. Entomol. Exp. Appl. 74:283–294.

Gould, F. 1983. Genetics of plant-herbivore systems: Interactions between applied and basic study, pp 599–653. *In*: Denno, R.F., M.S. McClure (Eds.), Variable Plants and Herbivores in Natural and Managed Systems, Academic Press.

Guo, Z., D. Chen, T. Schnurbusch. 2015. Variance components, heritability and correlation analysis of anther and ovary size during the floral development of bread wheat. J. Exp. Bot. 66(11): 3099–3111.

Gupta, A.K., R.K. Mittal, A. Ziauddin. 1999. Association and factor analysis in spring wheat. Ann. Agric. Res. 20: 481–485.

Gustafson, P., O. Raskina, X. Ma, E. Nevo. 2009. Wheat evolution, domestication and improvement, pp. 5–30. *In*: B.F. Carver (Ed.), Wheat science and trade, Wiley-Blackwell, Ames, IA.

Hardham, A.R., D.A., Jones, D. Takemoto. 2007. Cytoskeleton and cell wall function in penetration resistance. Curr. Opin. Plant Biol. 10: 342–348.

Harris, M.O., S. Rose, P. Malsch. 1993. The role of vision in the host plant-finding behavior of the Hessian fly. Physiol. Entomol. 18: 31–42.

Harris, M.O., M. Sandanayaka, W. Griffin. 2001. Oviposition preferences of the Hessian fly and their consequences for the survival and reproductive potential of offspring. Ecol. Entomol. 26: 473–86.

Harris, M.O., J.J. Stuart, M. Mohan, S. Nair, R.J. Lamb, O. Rohfritsch. 2003. Grasses and gall midges: Plant defense and insect adaptation. Annu. Rev. Entomol. 48:549–577.

Harris, M.O., T.P. Freeman, O. Rohfritsch, K.G. Anderson, S.A. Payne, J.A. Moore. 2006. Virulent Hessian fly (Diptera: Cecidomyiidae) larvae induce a nutritive tissue during compatible interactions with wheat. Ann. Entomol. Soc. Am. 99(2): 305–316.

Harris, M.O., T.P. Freemann, J.A. Moore, K.G. Anderson, S.A. Payne, K.M. Anderson, O. Rohfritsch. 2010. *H*-gene-mediated resistance to Hessian fly exhibits features of penetration resistance to fungi. Microscopy. 100(3): 279–289.

Hatchett, J. H., R.L. Gallun. 1970. Genetics of the ability of the Hessian fly, *Mayetiola destructor*, to survive on wheat having different genes for resistance. Ann. Entomol. Soc. Am. 63: 1400–1407.

Hatchett, J.H., G.L. Kreitner, R.J. Elzinga. 1990. Larval mouthparts and feeding mechanism of the Hessian fly (Diptera: Cecidomyiidae). Ann. Entomol. Soc. Am. 83: 1137–1147.

Heady, S.E., R.G. Lambert, C.V. Covell Jr. 1982. Determination of free amino acids in larval insect and gall tissues of the goldenrod, *Solidago Canadensis* L. Comp. Biochem. Physiol. 73: 641–644.

Heil, M. 2002. Ecological costs of induced resistance. Curr. Opin. Plant. Biol. 5: 345–350.

Herms, D.A., W.J. Mattson. 1992. The dilemma of plants: to grow or defend. Q. Rev. Biol. 67: 283–335.

Horber, E. 1972. Plant resistance to insects. Agric. Sci. Rev. 10: 1–11.

Hothorn, T., F. Bretz, P. Westfall. 2008. Simultaneous Inference in General Parametric Models. Biometrical Journal 50(3), 346–363.

Hothorn, T. 2015. Additional *multcomp* Examples. 1–11. <URL: https://cran.r-project.org/web/packages/multcomp/vignettes/multcomp-examples.pdf>.

Houle, G., G. Simard. 1996. Additive effects of genotype, nutrient availability and type of tissue damage on the compensatory response of Salix planifolia ssp. planifolia to stimulated herbivory. Oecologia. 107: 373–378.

Hudson, R.D., G.D. Buntin, D.J. Isenhour, E.F. Suber. 1991. Small Grain Insects *In* Summary of losses from insect damage and costs of control in Georgia. Douce, G.K. and R.M. McPherson (eds.). Univ. of G. Col. Of Agr. Exp. Stm. Special Pub. No. 64.

Irwin, D.L., L.W. Aarssen. 1996. Effects of nutrient level on cost and benefit of apical dominance in *Epilobium ciliatum*. Am. Midl. Nat. 136: 14–28.

Jankiewicz, L.S.; H. Plich, R. Antoszewski. 1970. Preliminary studies on the translocation of ^{14}C-labelled assimilates and $^{32}PO^{-3}$ towards the gall evoked by *Cynips (Diplolepis) quercusfolii* L. on oak leaves. Marcellia. 36: 163–172.

Karban, R., S.Y. Strauss. 1993. Effects of herbivores on growth and reproduction of their perennial host, *Erigeron glaucus*. Ecology. 74(1): 39–46.

Kennedy, G.G., F. Gould, O.M.B. Deponti, R.E. Stinner. 1987. Ecological, agricultural, genetic, and commercial considerations in the deployment of insect-resistant germplasm. Environ. Entomol. 16: 327–338.

Kirby, E.J.M., M. Appleyard. 1987. Cereal development guide, pp 1–85. Stoneleigh, Kenilworth, UK, NAC Cereal Unit.

Kosma, D.K., J.A. Nemacheck, M.A. Jenks, C.E. Williams. 2010. Changes in properties of wheat leaf cuticle during interactions with Hessian fly. The Plant Journal. 63: 31–43.

Lhaloui, S., L. Buschman, M. El Bouhssini, A. Amri, J.H. Hatchett, D. Keith, K. Starks, K. El Houssaini. 1992a. Infestations of *Mayetiola* spp. (Diptera: Cecidomyiidae) in bread wheat, durum wheat, and barley: Results of five annual surveys in the major cereal growing regions of Morocco. Al Awamia. 77: 21–53.

Lhaloui, S., L. Buschman, M. El Bouhssini, K. Starks, D. Keith, K. El Houssaini. 1992b. Control of *Mayetiola* species (Diptera: Cecidomyiidae) with carbofuran in bread wheat, durum wheat, and barley, with yield loss assessment and its economic analysis. Al Awamia. 77: 55–73.

Lhaloui S., J.H. Hatchett, G.E. Wilde. 1996. Evaluation of New Zealand barleys for resistance to *Mayetiola destructor* and *M. hordei* (Diptera: Cecidomyiidae) and the effect of temperature on resistance expression to Hessian fly. J. Econ. Entomol. 89: 562–67.

Li, C., M. Chen, S. Chao, J. Yu, G. Bai. 2013. Identification of a novel gene, *H34*, in wheat using recombinant inbred lines and single nucleotide polymorphism markers. TAG. 8: 2065–2071.

Liu, X.M., C.E. Williams, J.A. Nemacheck, H. Wang, S. Subramanyam, C. Zheng, M.S. Chen. 2010. Reactive oxygen species are involved in plant defense against a gall midge. Plant Physiol. 152: 985–999.

Mabry, C.M., P.W. Wayne. 1997. Defoliation of the annual herb *Abutilon theophrasti*: mechanisms underlying reproductive compensation. Oecologia. 111: 225–232.

Maghirang, E.B., G.L. Lookhart, S.R. Bean, R.O. Pierce, F. Xie, M.S. Caley, J.D. Wilson, B.W. Seabourn, M.S. Ram, S.H. Park, O.K. Chung, F.E. Dowell. 2006. Comparison of quality characteristics and breadmaking functionality of hard red winter and hard red spring wheat. Cereal Chem. 83(5): 520–528.

McColloch, J.W., H. Yuasa. 1917. Notes on the migration of the Hessian fly larvae. Anim. Behav. 7: 307–323.

McDonald, M., H. Ohm, K. Rinehart, M. Giovanini, C. Williams. 2014. *H33*: A wheat gene providing Hessian fly resistance for the Southeastern United States. Crop Science. 54: 2045–2053.

Menne, D. 2008. [R] general linear hypothesis glht() to work with lme(). <URL: https://stat.ethz.ch/pipermail/r-help/2008-January/151260.html>.

Meyer, G.A. 1993. A comparison of the impacts of leaf- and sap-feeding insects on growth and allocation of goldenrod. Ecology. 74(4): 1101–1116.

Mutkainen, P., M. Walls, A. Ojala. 1994. Sexual differences in responses to simulated herbivory in *Urtica dioica*. Oikos. 69: 397–404.

Mutkainen, P., M. Walls. 1995. Growth, reproduction and defense in nettles: responses to herbivory modified by competition and fertilization. Oecologia. 104: 487–495.

Nimchuk, Z., T. Eulgem, B.F. Holt III, J.L. Dangl. 2003. Recognition and responses in the plant immune system. Ann. Rev. Genet. 37: 579–609.

Olff, H., J. Van Andel, J.P. Bakker. 1990. Biomass and shoot/root allocation of five species from a grassland succession series at different combinations of light and nutrient supply. Funct. Ecol. 4: 193–200.

Painter, R.H. 1951. Insect Resistance in Crop Plants. Macmillan, New York, NY, USA.

Pedigo, L.P., S.H. Hutchins, L.G. Higley. 1986. Economic injury levels in theory and practice. Annu. Rev. Entomol. 31: 341–368.

Pedigo, L.P. 2002. Entomology and pest management. 3rd ed. Prentice Hall, Englewood Cliffs, NJ.

Peña, R.J. 2002. Wheat for bread and other foods. Bread Wheat: Improvement and Production. Rome: Food and Agriculture Organization of the United Nations.

Petcu, E., G. Petcu, C. Lazar, R. Vintila. 2003. Relationship between leaf area index, biomass and winter wheat yield obtained at Fundulea, under conditions of 2001 year. Rom. Agric. Res. 21–29.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, R Core Team. 2015. _nlme: Linear and Nonlinear Mixed Effects Models_. R package version 3.1-120, <URL: http://CRAN.R-project.org/package=nlme>.

Porter, D.R., M.O. Harris, L.S. Hesler, G.J. Puterka. 2009. Insects which challenge global wheat production, pp. 189–201. *In*: B. F. Carver (Ed.), Wheat science and trade, Wiley-Blackwell, Ames, IA.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. URL http://www.R-project.org/.

Ratcliffe, R.H., J.H. Hatchett. 1997. Biology and genetics of the Hessian fly and resistance in wheat, pp. 47–56. *In*: Bondarie, K. (Ed.), New Developments in Entomology, Research Signpost. Scientific Information Guild, Trivandrum, India.

Ratcliffe, R.H., S.E. Cambron, K.L. Flanders, M.A. Bosque-Peres, S.L. Clement, H.W. Ohm. 2000. Biotype composition of Hessian fly (Diptera: Cecidomyiidae) populations from the Southeastern, Midwestern, and Northwestern United States and virulence to resistant genes in wheat. J. Econ. Entomol. 93: 1319–1328.

Reese, J., J. Schwenke, P. Lamont, D. Zehr. 1994. Importance and Quantification of Plant Tolerance in Crop Pest Management Programs for Aphids: Greenbug Resistance in Sorghum. J. Agr. Entomol. 11: 255–270.

Refai, F.Y., E.T. Jones, B.S. Miller. 1955. Some biochemical factors involved in the resistance of the wheat plant to attack by the Hessian fly. Cereal Chemistry. 32: 437–442.

Richards, J.H. 1993. Physiology of plants recovering from defoliation, pp 85–93. Proc. XVII Int. Grasslands Congress, Palmerston North, New Zealand.

Rider Jr., S.D., W. Sun, R.H. Ratcliffe, J.J. Stuart. 2002. Chromosome landing near avirulence gene *vH13* in the Hessian fly. Genome. 45(5): 812–822.

Rohfritsch. O. 1992. Patterns in gall development. Biology of Insect-Induced Galls, pp. 60–86. Shorthouse, J., O. Rohfritsch (Eds.), Oxford University Press, New York, NY, USA.

Rosenthal, J.P., S.C. Welter. 1995. Tolerance to herbivory by a stemboring caterpillar in architecturally distinct maizes and wild relatives. Oecologia. 102: 146–155.

Sardesai, N., J. Nemacheck, S. Subramanyam, C.E. Williams. 2005. Identification and mapping of *H32*, a new wheat gene conferring resistance to Hessian fly. Theor. Appl. Genet. 111:1167–1173.

Schwachtje, J., P.E.H. Minchin, S. Jahnke, J.T. van Dongen, U. Schittko, U.I.T. Baldwin. 2006. SNF1-related kinases allow plants to tolerate herbivory by allocating carbon to roots. Proc. Natl. Acad. Sci. 103: 12935–12940.

Shah, W.A., H.U. Khan, S. Anwar, K. Nawab. 2011. Yield and yield components affected by different seed rates and nitrogen levels. Sarhad J. Agric. 27(1): 17–25.

Shukle, R.H., L.L. Murdock, R.L. Gallun. 1985. Identification and partial characterization of a major gut proteinase from larvae of the Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae). Insect Biochem. 15: 93–101.

Shukle, R. H., P.B. Grover, J.E. Foster. 1990. Feeding of Hessian fly (Diptera: Cecidomyiidae) larvae on resistant and susceptible wheat. Environ. Entomol. 19: 494–500.

Shukle R.H., P.B. Grover, G. Mocelin. 1992. Responses of susceptible and resistant wheat associated with Hessian fly (Diptera: Cecidomyiidae) infestation. Environ. Entom. 21: 845–853.

Shukle, R.H., S. Subramanyam, C.E. Williams. 2011. Effects of antinutrient proteins on Hessian Fly (Diptera: Cecidomyiidae) larvae. J. Insect. Physio. 26: 1–8.

Smedegaard-Petersen, V., O. Stølen. 1981. Effect of energy-requiring defense reactions on yield and grain quality in a powdery mildew-resistant barley cultivar. Phytopathology. 71: 396–399.

Smiley, R.W., J.A. Gourlie, R.G., Whittaker, S.A., Easley, K.K. Kidwell. 2004. Economic impact of Hessian fly (Diptera: Cecidomyiidae) on spring wheat in Oregon and additive yield losses with *Fusarium* crown rot and lesion nematode. J. Econ. Entomol. 97(2): 397–408.

Sosa, O., R.L. Gallun. 1973. Purification of races B and C of the Hessian fly by genetic manipulation. Ann. Entomol. Soc. Am. 66: 1065–1070.

Stern V.M., R. F. Smith, R. van den Bosch, K. S. Hagen. 1959. The integrated control concept. *Hilgardia*. 29: 81–101.

Strauss, S., A. Agrawal. 1999. The ecology and evolution of plant tolerance to herbivory. TREE. 14: 179–185.

Stuart, J.J., Chen, M.S., Harris, M., 2008. Hessian fly, pp. 93–100. *In*: Hunter, W., Kole, C. (Eds.), Genome Mapping and Genomics in Animals, vol. 1: Genome Mapping and Genomics in Arthropods. Springer, Berlin, Heidelberg, New York.

Subramanyam, S., N. Sardesai, D.P. Puthoff, J.M. Meyer, J.A. Nemacheck, M. Gonzalo, C. E. Williams. 2005. Expression of two wheat defense-response genes, *Hfr-1* and *Wci-1*, under biotic and abiotic stresses. Plant Sci. 170: 90–103.

Subramanyam, S., D.F. Smith, J.C. Clemens, M.A. Webb, N. Sardesai, C.E. Williams. 2008. Functional characterization of HFR1, a high-mannose *N*-glycan-specific wheat lectin induce by Hessian fly larvae. Plant Physiol. 147: 1412–1426.

Taylor, W.A. 1921. Varieties of winter wheat adapted to the Eastern United States. Farmer's Bulletin 1168, United States Department of Agriculture, WA.

Trumble, J.T., D.M. Kolodny-Hirsch, I.P. Ting. 1993. Plant compensation for arthropod herbivory. Annu. Rev. Entomol. 38: 93–119.

USDA. 2015. World Agricultural Supply and Demand Estimates.

Weis, A.E., R. Walton, C.L. Crego. 1988. Reactive plant tissue sites and the population biology of gallmakers. Ann. Rev. Entomol. 33: 467–486.

Wellso, S.G. 1991. Aestivation and phenology of the Hessian fly (Diptera: Cecidomyiidae) in Indiana. Environ. Entomol. 20: 795–801.

Welter, S.C., J.W. Steggall. 1993. Contrasting the tolerance of wild and domesticated tomatoes to herbivory: agroecological implications. Ecol. Appl. 3: 271–278.

Wickham, H. 2009. ggplot2: elegant graphics for data analysis. Springer, New York, NY.

Williams, C.E., Collier, C.C., Nemacheck, J.A., Chengzhi, L., Cambron, S.E., 2002. A lectin-like wheat gene responds systemically to attempted feeding by avirulent first-instar Hessian fly larvae. J. Chem. Ecol. 28: 1411–1428.

Wise, I.L., R.J. Lamb, R.I.H. McKenzie, J.W. Whistlecraft. 2006. Resistance to Hessian fly (Diptera: Cecidomyiidae) in a Canadian spring wheat cultivar. Can. Entomol. 638–646.

Wood, E.A., H.L. Chada, P.N. Saxena. 1969. Reaction of small grains and grain sorghum to three greenbug biotypes, pp. 1–5. Prog. Rep. Agric. State Univ. Okla. Stillwater, OK.

Yeats, T.H., K.J. Howe, A.J. Matas, G.J., Buda, T.W. Thannhauser, J.K.C. Rose. 2010. Mining the surface proteome of tomato (*Solanum lycopersicum*) fruit for proteins associated with cuticle biogenesis. J. Exp. Bot. 61: 3759–3771.

Zadok, J. C., T.T. Chang, C.F. Konzak. 1974. A decimal code for the growth stages of cereals. Weed Res. 14: 415–421.

Zhang, H.; K.M. Anderson; J. Reber; J.J. Stuart; S. Cambron; M.O. Harris. 2011. A Reproductive Fitness Cost Associated with Hessian Fly (Diptera: Cecidomyiidae) Virulence to Wheat's *H* Gene-Mediated Resistance. J. Econ. Entom. 104(3): 1055–1064.

CHAPTER 2: TOLERANCE AS A POTENTIAL CONTROL METHOD FOR HESSIAN FLY (DIPTERA: CECIDOMYIIDAE) IN WINTER WHEAT

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Accepted Pending Revision for *Crop Science*

CHAPTER 2. TOLERANCE AS A POTENTIAL CONTROL METHOD FOR HESSIAN FLY (DIPTERA: CECIDOMYIIDAE) IN WINTER WHEAT

2.1 Abstract

Tolerance in wheat may hold the key to reducing damage caused by the Hessian fly, *Mayetiola destructor*, while enabling the plant to grow normally and reducing the selection pressures leading to increased virulence, or resistance to wheat plant defenses, in fly populations. Susceptible Pioneer® brand variety 25R75, susceptible wheat cultivar 'Newton', and tolerant Pioneer® brand variety 25R78 were evaluated in two sets: 16 and 32 days post infestation (dpi). After infesting plants at the two to three leaf development stage with one Hessian fly per plant for three hours, larvae were allowed to develop until either 16 or 32 dpi, when leaf and tiller number, larvae number and size, leaf growth rates, and change in total and average leaf lengths were recorded.

At 16 dpi, while there were no significant differences in leaf number observed, infested tolerant plants exhibited significantly higher tiller numbers than uninfested plants. Additionally, total leaf length changes and leaf growth rates were significantly lower for infested tolerant plants versus uninfested plants. No permanent growth effects occurred in the 32-day set, and there were no significant differences in change in leaf length and leaf growth rate in infested tolerant plants compared to uninfested plants.

Infested tolerant plants exhibited significantly higher leaf numbers per plant, but no significant difference in tiller numbers compared to uninfested plants. No dead red larvae were observed, differing from resistant plants commonly used and preventing selection pressure on fly populations. Overall, tolerant plants had significantly smaller larval area than the susceptible lines. The absence of leaf or tiller loss, as well as leaf length and growth rate in Pioneer variety 25R78, could allow higher yields from this tolerant line due to the positive correlation of biomass and leaf area with yield (Petcu, 2003).

2.2 Introduction

Hessian flies, or *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae), are known as one of the most damaging wheat pests worldwide. One report estimated Hessian fly damage in the United States could reach $100 million per year (Cartwright and Jones, 1953). In 1989, Georgia alone experienced losses of $28 million dollars (Hudson et al., 1991). In 2004, a study in Oregon showed a 66 and 68% increase in grain yield for resistant genotypes over susceptible genotypes (Smiley et al., 2004). The susceptible genotypes produced two-thirds less grain than the resistant genotypes at intermediate infestation (50% or less infested plants) with a reduction of the grain quality (Smiley et al., 2004).

Severe crop loss due to Hessian fly infestations has been noted worldwide. For example, in Morocco, Hessian fly damage can lead to a complete crop loss, when infestations in the fall are high and occur during the more vulnerable younger crop stages (El Bouhssini, 1996). Yield loss of 42% can occur despite the use of insecticide Furadan

5G to control Hessian flies (Lhaloui et al., 1992a). Infested near susceptible and resistant isogenic lines of bread wheat (*Triticum aestivum* L.) demonstrated a 36% yield loss (Amri et al., 1992). Durum wheat (*T. turgidum* L. var. *durum*) was also studied, demonstrating a 32% yield loss due to Hessian fly infestation (Lhaloui et al., 1992b).

Research on the control of the Hessian fly on wheat (*Triticum aestivum* L. subsp. *aestivum*) has been primarily focused on resistance genes, or host *R* genes, which kill attacking larvae through antibiosis. Currently, 35 *R* genes, or *H* genes, have been identified: *H1–H34* and *Hdic* (Sardesai et al., 2005; Li et al., 2013; McDonald et al., 2014). However, selection pressure exerted on surviving flies leads to the increased frequency of new virulent biotypes capable of overcoming *R* genes. An increased frequency of the virulent biotypes can occur any time after *R* gene deployment, sometimes occurring immediately after the release of the line or within 5 – 10 years (Ratcliffe and Hatchett, 1997; Ratcliffe et al., 2000; Cambron et al., 2010).

For example, in the southeastern United States, biotypes exist that are virulent on wheat with *R* genes *H3, H5, H6,* and *H7H8* (Cambron et al., 2010). In that region, only six *R* genes (*H12, H18, H24, H25, H26,* and *H33*) are predicted to be effective against the fly populations (Cambron et al., 2010). Because of this constant race against Hessian fly virulence, various methods must be used to control Hessian fly. This includes releasing new *R* genes, planting after the “fly-free” date, and using insecticide-soaked seeds. However, these methods may not be the only control options. Another potential method of Hessian fly control may involve tolerance.

One definition of tolerance is the ability of the plant to continue growing despite insect attack (Reese et al., 1994). A second definition builds on the first by defining

tolerance in plants as the ability of the plant to recover in growth as well as potentially reproduce (Strauss and Agrawal, 1999). In contrast, plant resistance refers to any plant trait that can decrease the performance or preference of an herbivore (Rosenthal and Kotanen, 1994). This resistance may take two forms. The first is to avoid damage through escape (antixenosis) or defense (antibiosis) Antixenosis includes any method that affects the insect's ability to find, attack, feed on, or colonize a plant, while antibiosis refers to methods that harm, kill, or affect growth of the feeding stages of a pest (Painter, 1951). The second form of resistance is tolerance of damage according to Painter (1951). Tolerance may also include compensation, allowing regrowth, compared to a susceptible host with a similar population of pests (Painter, 1951). This third definition takes into account the role tolerance plays in plant resistance.

However, resistance in wheat to Hessian fly attack differs from general plant resistance and occurs through the *R-avr* interaction. This resistance causes larval death through its own form of antibiosis 3-5 days after insect attack without allowing growth and does not consist of antixenosis (Shukle et al., 1990). Unlike normal herbivore feeding where tissue damage occurs, Hessian fly larvae feed by altering the development and nutrient content of the epidermal cells under the leaf sheath. The damage does not occur directly to the leaves, but occurs within the leaf sheath of the plant where induced epidermal cells become permeable and rupture to form nutritive tissue (Shukle et al., 1992; Williams et al., 2011).

Each of the definitions of tolerance builds on the basic premise that tolerant plants can grow or suffer minimal yield impacts from insect attack without leading to a buildup of pest resistance. Tolerance is dependent on both the insect and the plant. Tolerance

relates to an insect's effect on a plant where a greater tolerance level results in less damage to the plant. Tolerance also relates to the plant's response to insect injury through recovery and growth (Reese et al., 1994).

One potential benefit of tolerance in any crop include the plant's ability to support and maintain natural enemy populations by not decreasing prey numbers through antixenosis (non-preference) or antibiosis (Horber, 1972). Tolerance could also increase economic injury levels, or the smallest number of pests to cause economic damage (Stern et al., 1959; Pedigo et al., 1986). This might reduce or delay required insecticide use (Reese et al., 1994). Thirdly, tolerance does not impose selection pressures that lead to the formation of novel biotypes (Reese et al., 1994; Strauss and Agrawal, 1999). The lack of selection pressure is due to the fact that tolerance is a plant reaction to insect attack with no direct effect on insect physiology, growth, reproduction, or biology (Reese et al., 1994).

Very little research has focused on tolerance in wheat to Hessian flies and there is scant research on partial tolerance. Marquillo hybrids, a cross of *R* gene *H18*-containing Marquillo and winter wheat, were found to be able to withstand Hessian fly infestation by surviving and providing yield even under heavy infestation (Agricultural Experiment Station Kansas State, 1940). This tolerance, when combined with poor larval survival, was a potent resistance trait. The wheat line 'Superb' can reduce yield loss from fly infestations by up to 65% compared to susceptible lines such as 'AC Barrie' due to the partial tolerance and antibiosis present in the line (Wise et al., 2006). These researchers considered tolerance as the ability of wheat stems to survive larval feeding without snapping (Wise et al., 2006). 'Superb' caused larval antibiosis which could place

selection pressure on fly populations. Pioneer variety 25R78 was screened by Sue Cambron in the USDA-ARS greenhouse in West Lafayette, IN. The screening demonstrated field tolerance. Flies continued to emerge from the plants after infestation, but plants did not die or stunt like susceptible plants. For this reason, Pioneer variety 25R78 (PI 619611) was chosen as the putative tolerant line to be studied in this experiment. Pioneer variety 25R75 was chosen to act as a susceptible control for this tolerant line.

It is theorized that tolerance in wheat might be used alongside resistance to help offset allocation expenses for *R* genes such as reduced yield, seed protein, and seed weight (Anderson et al., 2011; Smedegaard-Petersen and Stølen, 1981). Initially, there might be an allocation cost for a resistant and tolerant plant, but the cost would eventually disappear due to growth compensation (Anderson et al., 2011). This compensation could be completed via carbon deployment to larvae-inaccessible regions or redistribution of the carbon for growth after a pest departs (Schwachtje et al., 2006).

There are several reasons why tolerance to Hessian flies is believed to be present in wheat. First, susceptible ‘Newton’ has the ability to trigger growth through tiller production from an axillary coleoptile meristem in order to survive infestation (Anderson and Harris, 2006). Second, several infested resistant lines might demonstrate growth compensation through the production of superior qualities compared to uninfested plants when infested, such as greater seed numbers and heads as well as taller plants. For example, infested *H6* plants were taller, *H13* plants had a greater number of seeds and greater total seed weights, and *H9* and *H13* plants had more seed heads compared to susceptible lines (Anderson et al., 2011).

However, Hessian fly tolerance might not only be present in resistant and susceptible wheat lines, but could be a unique plant defense response by a wheat line. We asked whether or not the putative tolerant Pioneer® brand variety Pioneer variety 25R78 could survive Hessian fly infestation, continue to grow, and not directly kill the larvae via antibiosis. We also asked if leaf growth rates, leaf and tiller numbers, and leaf lengths would be able to recover from initial stunting. This would make the tolerant wheat line distinct from susceptible lines due to the lack of main tiller leaf growth in susceptible lines.

In an experiment performed by Anderson and Harris (2006), infested susceptible line 'Newton' demonstrated poor main tiller (stem) leaf growth with large growth deficits (shorter leaves) in all leaves as well as significant growth deficits in each individual leaf compared to uninfested plants. This growth was measured by measuring the length of each leaf from the lamina tip to either the ligule or to the leaf base (if the ligule was not yet formed and visible). Growth deficits would show smaller leaf lengths in either total leaf length or individual leaf lengths.

In the incompatible interactions, each leaf in the beginning showed growth effects for the region of greatest growth, evident in shorter third and fourth leaves. However, the fifth and main tiller leaves showed no growth deficits because their peak growth occurred after larvae had been killed in the *R* gene interaction (Anderson and Harris, 2006). Despite the initial growth deficits, total leaf lengths were similar or greater than those of uninfested plants, demonstrating that resistance can prevent serious or permanent growth deficits (Anderson and Harris, 2006). Normal growth or recovery of leaves of the main stem would signal a difference between the tolerant line Pioneer variety 25R78 and the

susceptible line ‘Newton’. No larvae death would signify a difference between Pioneer variety 25R78 and resistant lines.

The goal of this project was to investigate the growth effects of Hessian fly infestation on the tolerant Pioneer variety 25R78. This included analyzing the effects on leaf and tiller number as well as leaf growth rate, total leaf length, and individual leaf length. Tiller number was recorded to determine if larval feeding would stunt, kill, or limit tillers of tolerant plants, while individual leaf measurements were used since stunting of the third leaves can occur in compatible interactions and, occasionally, in incompatible interactions (Shukle, 1985; Hatchett et al., 1990). Total and individual leaf lengths were used to analyze fitness costs of resistance genes as well as growth effects of Hessian fly infestations (Anderson and Harris, 2006; Anderson and Harris, 2008). Combined, these measurements were used to analyze if the putative tolerant line could grow despite Hessian fly attack.

Tolerance has the potential to be a control method for the Hessian fly, as preliminary studies show that it does not entail fatal larval antibiosis, but still allows the plant to produce a yield that is not significantly different from that of resistant lines (Cambron, unpublished; Roe et al., unpublished). This combination would prevent selection pressure for new virulent biotype formation in fly populations. It was hypothesized that Pioneer variety 25R78 was tolerant to Hessian flies. The first prediction stated that infested putative tolerant Pioneer variety 25R78 plants would stunt, then recover the level of growth of the control plants. The second prediction stated that tolerant plants would not kill Hessian fly larvae. The hypothesis was tested by analyzing

effects of infestation on the growth of tolerant Pioneer® brand variety 25R78, susceptible Pioneer® brand variety 25R75, and susceptible variety ‘Newton’.

2.3 Materials and Methods

2.3.1 Plant Preparation

Pioneer wheat lines susceptible variety ‘Newton’, susceptible Pioneer® brand variety 25R75, and putative tolerant Pioneer® brand variety 25R78 were used. Pioneer variety 25R78 is identified as moderately resistant. This resistance is due to its potential field tolerance, originating from P2555 (PI 532914). Pioneer variety 25R78 has the following pedigree: Stella/2555 sib//3*2555/2510 sib/3/2571 and contains no known *R* genes. The exact parents of P2555 are unknown. However, the pedigree consists of one-quarter CIMMYT (International Maize and Wheat Improvement Center) spring wheat and three-quarters soft red winter wheat. Pioneer variety 25R75 (PI 614786) is a half sibling of Pioneer variety 25R78 with parent line 2571. ‘Newton’ is used as a commercial susceptible control since Pioneer variety 25R78 and 25R75 are currently not used commercially.

Two time intervals were used: 16 and 32 days post infestation (dpi). For each line and time interval, three pots were planted for the control (uninfested) and three pots were planted for the treatment (infested) with ten seeds sown in every pot, providing 30 seeds per line and treatment. Each wheat line was planted on different days under identical environmental conditions due to logistical constraints. The pots (10.16 cm. in diameter and 10.16 cm. in depth) were filled with soil to within 4 cm. of the rim and each pot was planted with 10 seeds pressed into the soil in a 3 cm. diameter circle. Two centimeters of

moistened soil was placed over the seeds. Planted seeds were watered with a solution of 2,300 mL and 5.48 g of fertilizer (Scotts General Purpose, 20:20:20). This solution was made twice and each pot was watered with 200 mL twice after planting for a total of 400 mL. The pots remained in the greenhouse at 19°C (± 5°C) under metal halide lamps (Hg; irradiance level 140 µmol m^{-2} s^{-1}) set at a photoperiod of 12 hours for 11 days after planting with watering as needed.

2.3.2 Insect Preparation

Hessian fly biotype E (avirulent/incompatible with wheat line *H3* 'Monon') was maintained by Sue Cambron at the USDA-ARS Crop Production and Pest Control Research Unit (West Lafayette, IN), according to the procedure described by Foster et al. (1988). While maintained, they were kept in 4°C cold storage with fluorescent lights. When ready to be used, wheat material with puparia was removed and placed in a clear plastic box (26 by 39 cm), moistened with a water-filled spray bottle, and kept under fluorescent lights at 18°C for 11 days until infestation.

2.3.3 Plant Infestation

On the 11th day after planting, the pots were taken from the greenhouse into the lab at 18°C with 80% (± 5%) humidity. Each plant had multiple measurements taken including leaf number, tiller number, and leaf length to the stem and to the soil. In order to keep plant measurements consistent, numbers 1–30 were written on the first leaf of each plant for each line and treatment using a black fine-tipped Sharpie marker. Each plant, including the controls, were covered with a clear, plastic 16 oz. cup (3 cm diameter

hole with mesh on top and 3–4 cm hole on side with a foam plug) (Fig. D.1). The plants were at the 2–3 leaf stage (Zadoks Scale = 12 or 13; Zadoks et al., 1974). Three gravid female and 1–2 male Hessian flies were placed inside each treated covered pot using an aspirator.

After 6 hours, all flies were removed and a Styrofoam plug was placed in the side hole. The pots were kept inside under a fluorescent light (irradiance at 18°C for 5 days. On the 5th day, the pots were placed in the growth chamber at 16°C (± 2°C) for 16 or 32 days with a photoperiod of 16:8 (day:night). Irradiance levels in the growth chamber (Percival with Intellus Control System) were approximately 650–700 µmol m^{-2} s^{-1}. Each pot was watered with 200 mL tap water 4–5 days per week. For the 32-day set, the pots were watered after 16 days in the growth chamber with a solution of 2,300 mL and 5.48 g of fertilizer (Scotts General Purpose, 20:20:20).

For the 16-day time interval, the pots were removed and measurements were taken of 20 plants per line and treatment. For the 32-day time interval, the pots were removed and measurements were taken of 30 plants per line and treatment. Any plants in the treatment group that had no eggs or larvae present were removed from the analysis as can be noted in the Statistical Analysis section.

2.3.4 Measurements taken

Due to the unexposed location of the larvae under the leaf sheath and the non-chewing mouthparts, measurements on plant effects do not include visible direct damage. Instead, measurements include plant growth effects. Measurements were made immediately before infestation and either 16 or 32 days after infestation. The

measurements for the 16 and 32 dpi sets included the following: leaf length to soil and to stem, tiller number, leaf number, and larvae number. Each plant measurement, except tiller number, was taken for only the main stem leaves. A leaf was measured if the leaf lamina was visible outside or above the whorl (Anderson and Harris, 2006).

Change in leaf length was calculated by subtracting the initial leaf measurement from the final leaf measurement and was an indicator of growth and leaf surface area. This was calculated for each leaf and the cumulative set of leaves for each plant to calculate the change in total leaf length measurement. Using a clear ruler pressed into the soil, the leaf height was measured from the tip of the leaf to the soil. Leaf height to stem was also measured from the leaf tip to the first ligule. Measurements were rounded down to the nearest millimeter. Individual and total leaf lengths were measured as was done by Anderson and Harris (2006).

The larval counts were destructively taken after pulling the leaf sheath away from the plant. Larvae were removed from the leaf sheath, placed a clear glass slides, and analyzed under an Olympus SZX16 stereomicroscope with an attached Olympus dp210 camera. Larvae pictures were taken using the Olympus cellSens software and larvae area was measured using the “polygon” tool on cellSens. Larvae were classified as “living” if they were white with no red pigment while “dead” larvae were classified as those that had not grown and showed red pigment (Zhang et al., 2011). Average area was calculated for each plant by calculating the sum of the area of every larva and dividing the sum by the total number of larvae for that plant.

Growth rate of the leaves at the stem was measured in several steps. First, each leaf was measured on the day of infestation and either 16 or 32 dpi. The leaf was

measured from the tip to the collar of the first ligule to control for movement of soil over time due to watering. The change in height was calculated by subtracting the initial measurement for each leaf from the final measurement. Then, this value was divided by either 16 or 32 to calculate the growth rate for each leaf, for the 16 or 32 day sets, respectively. The relative growth rate for each plant was calculated by finding the sum of each leaf growth rate. The growth rates of all the leaves of a given plant were summed together and divided by the total number of leaves. This resulted in the overall average growth rate of leaves per plant.

2.3.5 Statistical Analysis

Statistical analysis was performed in R (R Core Team, 2014). The design was a two-way factorial experiment with the following factors: wheat line and treatment (i.e. infested and uninfested). Wheat line had three levels: 'Newton', Pioneer variety 25R75, and Pioneer variety 25R78. Treatment had two levels: infested and uninfested. This design was done for two separate time segments: 16 and 32 days. Each time interval was analyzed separately. Three replicates of 10 plants were used for each level of treatment for each line. Plants that did not germinate, survive, or have successful infestation were removed and were not included in the measurements.

The final sample sizes for analysis in the 16-day set were the following: control Pioneer variety 25R78 (n = 23), infested Pioneer variety 25R78 (n = 23), control Pioneer variety 25R75 (n = 20), infested Pioneer variety 25R75 (n = 19), control 'Newton' (n = 20), and infested 'Newton' (n = 20). The sample sizes for analysis in the 32-day set were the following: control Pioneer variety 25R78 (n = 20), infested Pioneer variety 25R78 (n

= 18), control Pioneer variety 25R75 (n = 24), infested Pioneer variety 25R75 (n = 21), control ‘Newton’ (n = 26), and infested ‘Newton’ (n = 17). A statistical significance threshold of $\alpha = 0.05$ was used.

Leaf growth rate was a continuous variable with a normal distribution, change in total leaf length and average leaf length were continuous, ratio variables, and larvae number, leaf number, and tiller numbers were integer ratio scale variables. Each of these variables was analyzed using a mixed effect model with the pot number represented as a random variable nested within the treatment. The model was made using the *lme* function of package nlme in R (Piheiro et al., 2013; R Core Team, 2014). This was used to account for the experimental design of ten plants planted in every pot and helps resolve the non-independence of the plants within the same pot (Crawley, 2007). A post-hoc Tukey analysis was performed for pair-wise comparisons using the mixed effect model and the *glht* function of the multcomp package (Hothorn et al., 2008; Hothorn, 2015). Due to time and logistic constraints, the 16 and 32 day sets could not be conducted concurrently. Both time intervals contained the same experimental design and environmental conditions including growth chamber, soil type, temperature, and water source. No direct comparisons were made between the two time interval sets.

2.4 Results and Discussion

2.4.1 Leaf and Tiller Number

Leaf number is important as it reflects biomass and photosynthetic material. The leaves of the main stem were counted for both sets. At 16 dpi, there was no significant

difference in leaf number between the infested and uninfested tolerant Pioneer variety 25R78 plants (Z = 0.493, df = 111, P = 0.996) (Table 2.2; Fig. 2.2a). Infested plants of 'Newton' and Pioneer variety 25R75, however, showed significantly fewer leaves than their corresponding uninfested controls (P = 0.037 and P = < 0.001, respectively). Infested tolerant plants showed significantly more leaves than infested 'Newton' or Pioneer variety 25R75 (P = < 0.001 for both varieties) (Fig. 2.2a). At 32 dpi, infested Pioneer variety 25R78 plants showed a significantly greater number of leaves than uninfested plants (P = < 0.001) (Table 2.2; Fig. 2.2b). Infested plants of 'Newton' and Pioneer variety 25R75, however, showed significantly fewer leaves than their corresponding uninfested controls (P = < 0.001 for both varieties). Infested tolerant plants showed significantly more leaves than infested 'Newton' or Pioneer variety 25R75 (P = < 0.001 for both varieties) (Fig. 2.2b).

At 16 dpi, there were significantly more tillers for infested Pioneer variety 25R78 plants compared to uninfested plants (P = 0.003) (Table 2.3; Fig. 2.3a). Infested plants of Pioneer variety 25R75 (P = < 0.001) showed significantly fewer tillers than their corresponding uninfested controls, but there was no significant difference between infested and uninfested 'Newton' plants (P = 1.000). Infested tolerant plants showed significantly more tillers than infested 'Newton' or Pioneer variety 25R75 (P = < 0.001 for both varieties) (Fig. 2.3a). At 32 dpi, there was no significant difference in tiller number between the infested and uninfested tolerant Pioneer variety 25R78 plants (P = 1.000) (Table 2.3; Fig. 2.3b). Infested plants of Pioneer variety 25R75 showed significantly fewer leaves than their corresponding uninfested controls (P = < 0.001), but there was no difference between infested and uninfested 'Newton' plants (P = 1.000).

There was no significant difference between infested tolerant plants and infested ‘Newton’ or Pioneer variety 25R75 (P = 0.190 and P = 0.095, respectively) (Fig. 2.3b).

2.4.2 Change in Total Leaf Length

At 16 dpi, infested plants showed significantly smaller total leaf lengths compared to uninfested plants for Pioneer variety 25R78, Pioneer variety 25R75, and ‘Newton’ (P = < 0.001 for all varieties) (Table 2.4; Fig. 2.5a). Infested tolerant plants had significantly greater total leaf lengths than infested plants of ‘Newton’ and Pioneer variety 25R75 (P = < 0.001 for both varieties) (Fig. 2.5a).

At 32 dpi, however, there was no significant difference in total leaf length change between infested and uninfested plants for Pioneer variety 25R78 (Z = -0.681, P = 0.984). Significant differences remained for infested and uninfested plants of Pioneer variety 25R75 (Z = -18.30, P = < 0.001) and ‘Newton’ (Z = -15.23, P = < 0.001) (Table 2.4; Fig. 2.5b). Infested tolerant plants had significantly greater total leaf lengths than infested plants of ‘Newton’ (Z = 13.63, P = < 0.001) and Pioneer variety 25R75 (Z = 13.89, P = < 0.001) (Fig. 2.5b).

2.4.3 Individual Leaf Length Changes

Infested tolerant Pioneer variety 25R78 plants had significantly smaller leaf length changes at 16 dpi for the third leaf length than uninfested plants (P = < 0.001) (Table 2.5; Fig. 2.6c). Infested Pioneer variety 25R78 plants had significantly smaller leaf lengths at 32 dpi for the third and fourth leaf lengths than uninfested plants (P = < 0.001 for both leaves) (Table 2.5; Figs. 2.7c and 2.7d). However, the infested Pioneer variety

25R78 plants showed a significantly greater leaf length for the sixth leaf at 32 dpi compared to the uninfested plants (P = < 0.001) (Table 2.5; Fig. 2.7f).

Infested Pioneer variety 25R75 showed significantly smaller leaf lengths for each leaf (P = < 0.001 for each leaf) except the second leaf at 16 dpi (P = 0.833) (Table 2.5; Fig. 2.6). At 32 dpi, infested Pioneer variety 25R75 showed significantly smaller leaf lengths for each leaf (P = < 0.001, P = 0.013, P = < 0.001, P = < 0.001, and P = < 0.001, respectively), but the sixth leaf (P = 0.504) (Table 2.5; Fig. 2.7). At 16 dpi, 'Newton' showed significant stunting due to infestation in the second and third leaves (P = < 0.001 and P = < 0.001, respectively). The first, fourth, fifth, and sixth leaves did not show this significant stunting because of the presence of only one plant in the set that had six leaves (Table 2.5; Fig. 2.6). At 32 dpi, 'Newton' showed no difference for the first leaf (P = 0.999), but significant differences for the other leaves (P = 0.002, P = < 0.001, P = < 0.001, P = < 0.001, and P = < 0.001, respectively) (Table 2.5; Fig. 2.7).

2.5 Discussion

Tolerant plants do appear to affect size and number of larvae (Appendix A; Table 2.1; Fig. 2.1). Infested tolerant plants had significantly fewer larvae than 'Newton' at 16 dpi, and significantly fewer larvae than both susceptible lines at 32 dpi. In the 16-day set, infested tolerant plants had significantly smaller larvae compared to 'Newton', and significantly smaller larvae compared to both susceptible lines in the 32-day set (Appendix A; Table 2.1; Figs. 2.1a and 2.1b). However, none of the larvae were dead 1st-instars, known as dead red larvae. Dead red larvae are found on resistant plants in incompatible interactions. Larval growth appeared to be reduced by the tolerant plants.

This could be considered antibiosis according to Painter (1951). Also, the presence of no dead red larvae potentially demonstrates that larval antibiosis was incomplete unlike the antibiosis incurred by resistant plants where the majority of larvae are killed before they grow (Shukle et al., 1990). This indicates that the putative tolerant Pioneer variety 25R78 does not kill larvae. However, the presence of significantly smaller larvae might indicate some antibiotic effect unrelated to *R* genes that might affect development. Despite this, no larval death might prevent any selection pressures on the fly population.

Tolerant plants might have smaller larvae due to the continued growth of the plant, leading to a process where larvae are pushed outside of the leaf sheath or away from their feeding site. Also, the plants may produce lectins that affect the insect, or the plants may reduce the feeding site's ability to act as nutritive tissue (Shukle et al., 2011). The ability to affect growth or size of larvae without larval death requires further study to understand whether this is a direct or indirect ability.

Although there was no significant difference in leaf number for Pioneer variety 25R78 at 16 dpi, there was an increase in leaf number at 32 dpi, which could indicate the potential for superior qualities including more heads and seeds, faster reproduction, and increased biomass (Table 2.2; Figs. 2.2a and 2.2b) (Anderson et al., 2011). Previous research has shown infested susceptible 'Newton' plants have severe growth deficits in main tiller/stem leaves (Anderson and Harris, 2006). This study confirmed the negative impact of Hessian fly infestation on the two susceptible lines used, but demonstrated the potential of the tolerant line to counteract growth effects caused by infestation.

Leaf and tiller number are closely related so that an increase in tillering rate and leaf emergence rate can increase leaf number (Friend, 1965). A greater number of tillers

can increase leaf number and emergence. Tillers also have the potential to produce fertile heads, increasing yield. For example, reports from Nerson (1980) indicated an increase in wheat yield of at least double as tiller number increased. At 16 dpi, there were significantly more tillers for infested tolerant Pioneer variety 25R78 plants compared to the uninfested plants ($P = 0.003$) (Table 2.3; Fig. 2.3a). There was no significant difference in tiller number at 32 dpi (Table 2.3; Fig. 2.3b). Even though the 32-day set showed fewer tillers than the 16-day set, the data indicates that the tolerant line does not appear to have lost tillers. Tiller numbers are, at the very least, equivalent between infested and uninfested plants, with no loss in tillers.

Despite the difference in tiller number between the two sets, infested tolerant plants showed significantly more tillers than the infested susceptible plants ($P = < 0.001$ for both varieties) (Fig. 2.3). This could indicate that Pioneer variety 25R78 may demonstrate tolerance in a different way than the increased tiller number proposed by Anderson and Harris (2006). However, susceptible line ‘Newton’ and susceptible Pioneer variety 25R75 did not show any increase in tiller number in either of the two sets. The infested tolerant plants did show a slight, but significant, increase in tiller numbers compared to the uninfested tolerant plants for 16 dpi. However, the 32-day set showed no significant difference in tiller number between the uninfested and the infested tolerant plants. The absence of tiller loss is important for head production, yield production, and leaf emergence (Friend, 1965; Nerson, 1980).

Plants of each infested line in the 16-day set showed a significantly smaller relative leaf growth rate than the corresponding uninfested plants (Appendix A; Table 2.4; Fig. 2.4a). In the 32-day set, the infested and uninfested tolerant plants showed no

significant difference at a p-value of 0.05. However, plants from both infested susceptible lines had significantly smaller leaf growth rates compared to their corresponding uninfested plants. The 32-day set indicated that tolerant plants have slightly smaller, but statistically insignificant, leaf growth rates compared to uninfested plants. Although there was a small difference in tolerant plants, the leaf growth rates for infested tolerant plants were significantly greater than those of infested plants for both susceptible lines. This indicates that tolerance might be able to reduce growth effects compared to susceptible lines by reducing effects on leaf growth rate.

Change in total leaf lengths was used as a measurement of plant growth effects according to Anderson and Harris (2006) who used total leaf lengths as a measure of growth deficits. Infested Pioneer variety 25R78 showed significantly smaller total leaf length changes compared to uninfested plants at 16 dpi (Table 2.4; Fig. 2.5a). However, by 32 dpi, significantly greater total leaf growth was observed for infested plants compared to uninfested tolerant plants (Table 2.4; Fig. 2.5b). The smaller growth rate at 16 dpi suggests potential stunting of the infested tolerant plants compared to the uninfested plants. The larval feeding appeared to affect the growth of the leaves. However, the infested plants showed significantly greater changes in leaf length compared to the infested Pioneer variety 25R75 and ‘Newton’ plants. Leaf growth was suppressed less for the infested tolerant plants compared to their infested susceptible counterparts (Figs. 2.5a and 2.5b). In the 32-day set, there was no significant difference between leaf length of infested and uninfested tolerant plants (Table 2.4; Fig. 2.5b). Similar to the 16-day set, the infested plants showed significantly greater changes in leaf length compared to the infested Pioneer variety 25R75 and ‘Newton’ plants.

Each of the leaves measured was part of the main stem or tiller. The tolerant line evidenced growth in the main stem with a greater number of leaves and leaf growth and similar leaf heights versus the leaves of the uninfested plants. Infested tolerant Pioneer variety 25R78 may show initial stunting and growth effects on the third leaf for both 16 and 32 dpi and the fourth leaf for 32 dpi based on their significantly smaller leaf lengths compared to uninfested plants (Table 2.5; Figs. 2.6c, 2.7c, and 2.7d). However, there was no significant difference in leaf length for the first or second leaves for 16 and 32 dpi (Table 2.5; Figs. 2.6a, 2.6b, 2.7a, and 2.7b). There was also no significant difference in leaf length for the fifth leaf for 32 dpi (Table 2.5; Fig. 2.7e). The leaf length for the sixth leaf was significantly greater for the infested Pioneer variety 25R78 plants than the uninfested Pioneer variety 25R78 plants since uninfested plants having more than five leaves were uncommon (Table 2.5; Fig. 2.7f).

Several resistant wheat lines have been reported to suffer initial stunting for the third and fourth leaves similar to what has been observed for the tolerant Pioneer variety 25R78, but not for the two susceptible lines ‘Newton’ and Pioneer variety 25R75. A previous study analyzed the growth effects of larval feeding for lines *H9*, *H13*, and *H6* using the sampling times of 36, 156, and 348 hours and measuring total and individual leaf lengths (Anderson and Harris, 2006). It was concluded that *R* genes cannot stop larvae from affecting the leaf growth zones and that the effects on leaf growth can be systemic (Anderson and Harris, 2006). However, despite the stunted third and fourth leaves, the fifth and main tiller leaves showed quick growth due to availability of resources within the plant (Anderson and Harris, 2006). Somehow, tolerant plants appear

to escape with growth deficits in only the third leaf at 16 dpi and the third and fourth leaves at 32 dpi, even though larval death was not observed.

With a successful feeding site and no *R-Avr* gene interaction, larval effectors should be affecting growth and development of the plant longer than just the third and fourth leaves without allowing plant recovery. It is unknown how the plant can accomplish recovery in growth. However, Pioneer variety 25R78 fits the definition of tolerance which describes plant recovery and growth despite insect attack. Greater leaf growth and production is important for greater grain yield. Grain yield, as suggested by a study by Benbi (1994), was determined by the maximum leaf area index.

The hypothesis in Section 2.2 stated that Pioneer variety 25R78 was tolerant to Hessian flies. This hypothesis appeared to be supported by the results. Stunting did not occur in leaf or tiller numbers. Tolerance appears to prevent loss in leaf or tiller number for both time intervals with an increase in leaves for the 32-day set. Unfortunately, using two separate plant sets for 16 and 32 dpi prevented direct comparisons between the two. Although the 16-day set showed stunting through significantly smaller changes in total leaf length and individual leaf lengths as well as smaller leaf growth rates, the 32-day set showed no significant difference in these measurements. This might be due to the duration of the study with effects differing between 16 or 32-measurements. These results indicate the potential for stunting in these measurements, but also the potential for growth comparable to uninfested plants.

Despite the significantly smaller measurements for the 16-day set, infested tolerant plants for both time intervals showed significantly smaller negative growth effects for almost every measurement compared to infested susceptible plants for both

lines with the exception of the leaf growth rate at 16 dpi. These results are important for key long-term outcomes such as yield and economic impact. An increase in leaf number, total leaf length, and individual leaf length results in a greater plant biomass, larger leaf area, and potentially greater yield compared to plants with fewer or shorter leaves. Biomass and leaf area index are positively correlated until the anthesis phase while yield and biomass of certain winter wheat lines are positively correlated (Petcu, 2003). Biomass, yield, number of ears/m^2, and number of seeds are positively correlated (Petcu, 2003). The reduced growth effects on tolerant plants could result in greater yield due to reduced effects on biomass and leaf area.

Several benefits of tolerance include the plant's ability to survive and grow despite being infested. This could lead to increased yield production without harming the larvae through larval antibiosis associated with *R* genes. There were no dead larvae observed on infested plants of all three lines, indicating lack of the type of antibiosis caused by *R* gene interactions. Without antibiosis, there should be no selection pressure and ultimately no new biotype formation. Another advantage is the possibility that the plant growth could push the larvae out of the leaf sheath and into the environment. This would make the larvae susceptible to desiccation and predation, potentially reducing fly populations on the individual plant and in the field, while not directly harming the larvae. Future research will analyze the effect of plant growth on larvae and yield effects of infestation.

2.6 Conclusion

The results of this study partially support the hypothesis stated in the Introduction of this chapter. The hypothesis stated that Pioneer variety 25R78 was tolerant to Hessian flies. If the line was tolerant, it would be predicted that the line would not directly kill the larvae, but grow despite initial stunting. In this study, there were no dead larvae observed on tolerant plants in either time interval, supporting the hypothesis. However, the larvae were significantly smaller compared to the larvae on the two susceptible lines, suggesting that some type of antibiosis might be occurring, other than the fatal antibiosis caused by *R* genes. However, the presence of smaller larvae might be due to plant growth affecting the position of larvae in relation to their feeding site. This possibility will be addressed in Chapter 3.

Additionally, the hypothesis was supported by Pioneer variety 25R78 plant growth. The results of the individual leaf length measurements for infested plants indicate that stunting did occur in the third leaf in the 16-day set and the third and fourth leaf in the 32-day set, but no significant differences in the other leaves compared to uninfested plants. No loss in leaf or tiller number indicates no permanent stunting. There were negative growth effects in leaf growth rate and change in total leaf length on tolerant plants for the 16-day set, with no negative growth effects for these measurements for the 32-day set. In conclusion, the observation of no larval death as well as the ability to recover from stunting indicates that tolerant Pioneer variety 25R78 could be a useful mechanism of control of Hessian fly populations through the plant's survival and lack of larval antibiosis.

2.7 References

Agricultural Experiment Station Kansas State College of Agriculture and Applied Science. 1940. Transference of Hessian Fly Resistance and Other Characteristics of Marquillo Spring Wheat to Winter Wheat. Technical Bulletin No. 49. Kansas State Printing Plant. Topeka, KS.

Anderson, K.G., M.O. Harris. 2006. Does *R* gene resistance allow wheat to prevent plant growth effects associated with Hessian Fly (Diptera : Cecidomyiidae) attack? Plant Resistance. 99(5): 1842–1853.

Anderson, K.M, Q. Kang, J. Reber, M.O. Harris. 2011. No fitness cost for wheat's *H* gene-mediated resistance to Hessian Fly (Diptera: Cecidomyiidae). J. Econ. Entom. 104(4): 1393–1405.

Benbi, D.K. 1994. Prediction of leaf area indices and yields of wheat. J. Agric. Sci. 122: 13–20.

Cambron, S.E., G.D. Buntin, R. Weisz, J.D. Holland, K.L. Flanders, B.J. Schemerhorn, R.H. Shukle. 2010. Virulence in Hessian fly (Diptera: Cecidomyiidae) field collections from the southeastern United States to 21 resistance genes in wheat. J. Econ. Entomol. 103: 2229–2235.

Cartwright, W.B., E.T. Jones. 1953. The Hessian fly and how losses from it can be avoided, pp. 1–9. USDA Farmers' Bull. 162.

Crawley, M.J. 2007. Mixed Effects Models, pp. 630–637. *In* M.J. Crawley (ed.), The R Book. John Wiley & Sons, Ltd., West Sussex, United Kingdom.

El Bouhssini, M., S. Lhaloui, A. Amri, M. Jlibene, J.H. Hatchett, N. Nssarellah, M. Nachitt. 1996. Wheat genetic control of Hessian fly (Diptera: Cecidomyiidae) in Morocco. Field Crops Res. 45: 111–114.

Friend, D.J.C. 1965. Tillering and leaf production in wheat as affected by temperature and light intensity. Canadian Journal of Botany. 43(9): 1063–1076.

Foster, J.E., J.E. Araya, G.G. Safranski, S.E. Cambron, P.L. Taylor. 1988. Rearing Hessian fly for use in the development of resistant wheat. Purdue Univ. Argic. Exp. Stn. Bull. 536: 1–16.

Horber, E. 1972. Plant resistance to insects. Agric. Sci. Rev. 10: 1–11.

Hothorn, T., F. Bretz, P. Westfall. 2008. Simultaneous inference in general parametric models. Biometrical J. 50(3): 346–363.

Hothorn, T. 2015. Additional *multcomp* Examples. 1-11. <URL: https://cran.r-project.org/web/packages/multcomp/vignettes/multcomp-examples.pdf>.

Hudson, R.D., G.D. Buntin, D.J. Isenhour, E.F. Suber. 1991. Small Grain Insects. *In*: Douce, G.K., R.M. McPherson (Eds.), Summary of losses from insect damage and costs of control in Georgia, Univ. of G. Col. Of Agr. Exp. Stm. Special Pub. No. 64.

Lhaloui, S., L. Buschman, M. El Bouhssini, A. Amri, J.H. Hatchett, D. Keith, K. Starks, K. El Houssaini. 1992a. Infestations of *Mayetiola* spp. (Diptera: Cecidomyiidae) in bread wheat, durum wheat, and barley: Results of five annual surveys in the major cereal growing regions of Morocco. Al Awamia. 77: 21–53.

Lhaloui, S., L. Buschman, M. El Bouhssini, K. Starks, D. Keith, K. El Houssaini. 1992b. Control of *Mayetiola* species (Diptera: Cecidomyiidae) with carbofuran in bread wheat, durum wheat, and barley, with yield loss assessment and its economic analysis. Al Awamia. 77: 55–73.

Li, C., M. Chen, S. Chao, J. Yu, G. Bai. 2013. Identification of a novel gene, *H34*, in wheat using recombinant inbred lines and single nucleotide polymorphism markers. TAG. 8: 2065–2071.

McDonald, M., H. Ohm, K. Rinehart, M. Giovanini, C. Williams. 2014. *H33*: A wheat gene providing Hessian Fly resistance for the southeastern United States. Crop Science. 54: 2045–2053.

Menne, D. 2008. [R] general linear hypothesis glht() to work with lme(). <URL: https://stat.ethz.ch/pipermail/r-help/2008-January/151260.html>.

Nerson, H., 1980. Effects of population density and number of ears on wheat yield and its components. Field Crops Res. 3: 225-234.

Pedigo, L.P., S.H. Hutchins, L.G. Higley. 1986. Economic injury levels in theory and practice. Annu. Rev. Entomol. 31: 341–368.

Petcu, E., G. Petcu, C. Lazar, R. Vintila. 2003. Relationship between leaf area index, biomass and winter wheat yield obtained at Fundulea, under conditions of 2001 year. Rom. Agric. Res. 21–29.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, R Core Team. 2015. _nlme: Linear and Nonlinear Mixed Effects Models_. R package version 3.1-120, <URL: http://CRAN.R-project.org/package=nlme>.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. URL http://www.R-project.org/.

Ratcliffe, R.H., J.H. Hatchett. 1997. Biology and genetics of the Hessian fly and resistance in wheat, pp. 47–56. *In*: Bondarie, K. (Ed.), New Developments in Entomology, Research Signpost. Scientific Information Guild, Trivandrum, India.

Ratcliff, R.H., S.E. Cambron, K.L. Flanders, N.A. Bosque-Perez, S.L. Clement, H.W. Ohm. 2000. Biotype composition of Hessian fly (Diptera: Cecidomyiidae) populations from the Southeastern, Midwestern, and Northwestern United States and virulence to resistance genes in wheat. J. Econ. Entomol. 93: 1319–1328.

Reese, J., J. Schwenke, P. Lamont, D. Zehr. 1994. Importance and quantification of plant tolerance in crop pest management programs for aphids: greenbug resistance in sorghum. J. Agr. Entomol. 11: 255–270.

Sardesai, N., J. Nemacheck, S. Subramanyam, C.E. Williams. 2005. Identification and mapping of H32, a new wheat gene conferring resistance to Hessian fly. Theor. Appl. Genet. 111:1167–1173.

Schwachtje, J., P.E.H. Minchin, S. Jahnke, J.T. van Dongen, U. Schittko, U.I.T. Baldwin. 2006. SNF1 related kinases allow plants to tolerate herbivory by allocating carbon to roots. Proc. Natl. Acad. Sci. 103: 12935–12940.

Shukle R.H., P.B. Grover, G. Mocelin. 1992. Responses of susceptible and resistant wheat associated with Hessian fly (Diptera: Cecidomyiidae) infestation. Environ. Entom. 21: 845–853.

Shukle, R.H., S. Subramanyam, C.E. Williams. 2011. Effects of antinutrient proteins on Hessian Fly (Diptera: Cecidomyiidae) larvae. J. Insect. Physio. 26: 1–8.

Smedegaard-Petersen, V., O. Stølen. 1981. Effect of energy-requiring defense reactions on yield and grain quality in a powdery mildew-resistant barley cultivar. Phytopathology. 71: 396–399.

Smiley, R.W., J.A. Gourlie, R.G. Whittaker, S.A. Easley, K.K. Kidwell. 2004. Economic impact of Hessian Fly (Diptera: Cecidomyiidae) on spring wheat in Oregon and additive yield losses with Fusarium crown rot and lesion nematode.” J. Econ. Entomol. 97(2): 397–408.

Stern V.M., R.F. Smith, R. van den Bosch, K.S. Hagen. 1959. The integrated control concept. *Hilgardia*. 29: 81–101.

Strauss, S., A. Agrawal. 1999. The ecology and evolution of plant tolerance to herbivory. TREE. 14: 179–185.

USDA. 2015. World Agricultural Supply and Demand Estimates.

Wickham, H. 2009. ggplot2: elegant graphics for data analysis. Springer-Verlag New York, NY.

Welter, S.C., J.W. Steggall. 1993. Contrasting the tolerance of wild and domesticated tomatoes to herbivory: agroecological implications. Ecol. Appl. 3: 271–278.

Williams, C.E., J.A. Nemacheck, J.T. Shukle, S. Subramanyam, K.D. Saltzmann, R.H. Shukle. 2011. Induced epidermal permeability modulates resistance and susceptibility of wheat seedlings to herbivory by hessian fly larvae. J. Exp. Bot. 62(12): 4521–4531.

Wise, I.L., R.J. Lamb, R.I.H. McKenzie, J.W. Whistlecraft. 2006. Resistance to Hessian fly (Diptera: Cecidomyiidae) in a Canadian spring wheat cultivar. Can. Entomol. 638–646.

Zadoks, J.C., T.T. Chang, C.F. Konzak. 1974. A decimal code for the growth stages of cereals. Weed Sci. 14: 415–421.

Zhang, H.; K.M. Anderson; J. Reber; J.J. Stuart; S. Cambron; M.O. Harris. 2011. A Reproductive Fitness Cost Associated with Hessian Fly (Diptera: Cecidomyiidae) Virulence to Wheat's *H* Gene-Mediated Resistance. J. Econ. Entom. 104(3): 1055–1064.

2.8 Tables and Figures

Table 2.1 Analysis of the larval area means between treatments of each wheat lines as determined by a mixed effects model.

Wheat Line	Z value		P value	
	16 dpi†	32 dpi	16 dpi	32 dpi
Pioneer 25R78-'Newton'	-0.350	-2.425	0.935	0.041*
Pioneer 25R78- Pioneer 25R75	-4.588	-3.078	< 0.001***	0.006**
Pioneer 25R75- 'Newton'	4.421	0.396	< 0.001***	0.917

* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$.
† dpi: days post infestation

Table 2.2 Mean leaf number with the results of an ANOVA analysis using a mixed effects model, determining the effect of wheat line, infestation, and their interaction on leaf number.

Wheat Line	Treatment	Mean (Standard Deviation)		Z value		P value	
		16 dpi†	32	16	32	16	32
'Newton'	C‡	3.500 (0.513)	4.923 (0.272)	-2.952	-17.73	0.037*	< 0.001*
	T§+	3.150 (0.671)	3.000 (0)				
Pioneer variety 25R75	C	4.000(0)	5.083 (0.504)	-8.325	-21.84	< 0.001*	< 0.001*
	T	3.000 (0)	3.000(0)				
Pioneer variety 25R78	C	3.900 (0.308)	5.200 (0.410)	0.493	7.177	0.996	< 0.001*
	T	3.957 (0.209)	5.944 (0.236)				

* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$.
† dpi: days post infestation
‡ 'C': indicates control or uninfested plants.
§'T': indicates Hessian fly-infested plants.

Table 2.3 Mean tiller number with the results of an ANOVA analysis using a mixed effects model, determining the effect of wheat line, infestation, and their interaction on tiller number.

Wheat Line	Treatment	Mean (Standard Deviation)		Z value		P value	
		16 dpi†	32 dpi	16 dpi	32 dpi	16 dpi	32 dpi
'Newton'	C‡	1.050 (0.224)	1.000 (0)	0.000	-0.029	1.000	1.000
	T§	1.050 (0.224)	1.000 (0)				
Pioneer variety 25R75	C	1.900 (0.478)	1.670 (0.637)	-5.476	-4.984	< 0.001***	< 0.001***
	T	1.000 (0)	1.000 (0)				
Pioneer variety 25R78	C	1.500 (0.607)	1.400 (0.598)	3.742	0.083	0.003**	1.000
	T	2.087 (0.900)	1.389 (0.598)				

* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$.
† dpi: days post infestation
‡ 'C': indicates control or uninfested plants.
§ 'T': indicates Hessian fly-infested plants.

Table 2.4 A post-hoc Tukey analysis using the mixed effects model for leaf number, tiller number, leaf growth rate, and total leaf length change.

	Wheat Line	Z value		P value	
		16 dpi†	32 dpi	16 dpi	32 dpi
Leaf Growth Rate	'Newton'	-8.272	-14.36	< 0.001***	< 0.001***
	25R75	-11.75	-15.83	< 0.001***	< 0.001***
	25R78	-7.435	-2.660	< 0.001***	0.082
Total Leaf Length	'Newton'	-7.163	-15.23	< 0.001***	< 0.001***
	25R75	-13.75	-18.30	< 0.001***	< 0.001***
	25R78	- 6.565	-0.681	< 0.001***	0.984

* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$.
† dpi: days post infestation

Table 2.5 Differences in individual leaf lengths between infested and uninfested plants for each wheat line using a post-hoc Tukey analysis of a mixed effects model and represented as a Z-value (P-value).

	First	Second	Third	Fourth	Fifth	Sixth
Wheat Line			16 Days			
Pioneer variety 25R78	-0.761 (0.974)	-2.615 (0.092)	-7.602 (< 0.001) ***	7.672 (0.768)		
Pioneer variety 25R75	-5.393 (< 0.001) ***	-1.090 (0.883)	-10.66 (< 0.001) ***	-10.57 (< 0.001) ***		
'Newton'	-3.125 (0.022)*	-4.711 (< 0.001) ***	-6.171 (< 0.001) ***	-2.077 (0.284)	1.747 (.500)	1.747 (0.500)
Wheat Line			32 Days			
Pioneer variety 25R78	-0.961 (0.930)	-0.016 (1.000)	-4.510 (< 0.001) ***	-5.075 (< 0.001) ***	0.999 (0.916)	8.686 (< 0.001) ***
Pioneer variety 25R75	-4.945 (< 0.001) ***	-3.246 (0.013)*	-12.83 (< 0.001) ***	-17.73 (< 0.001) ***	-9.285 (< 0.001) ***	-1.738 (0.504)
'Newton'	-0.181 (0.999)	-3.508 (0.001)***	-16.48 (< 0.001) ***	-14.75 (< 0.001) ***	-5.689 (< 0.001) ***	

* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$.
† dpi: days post infestation

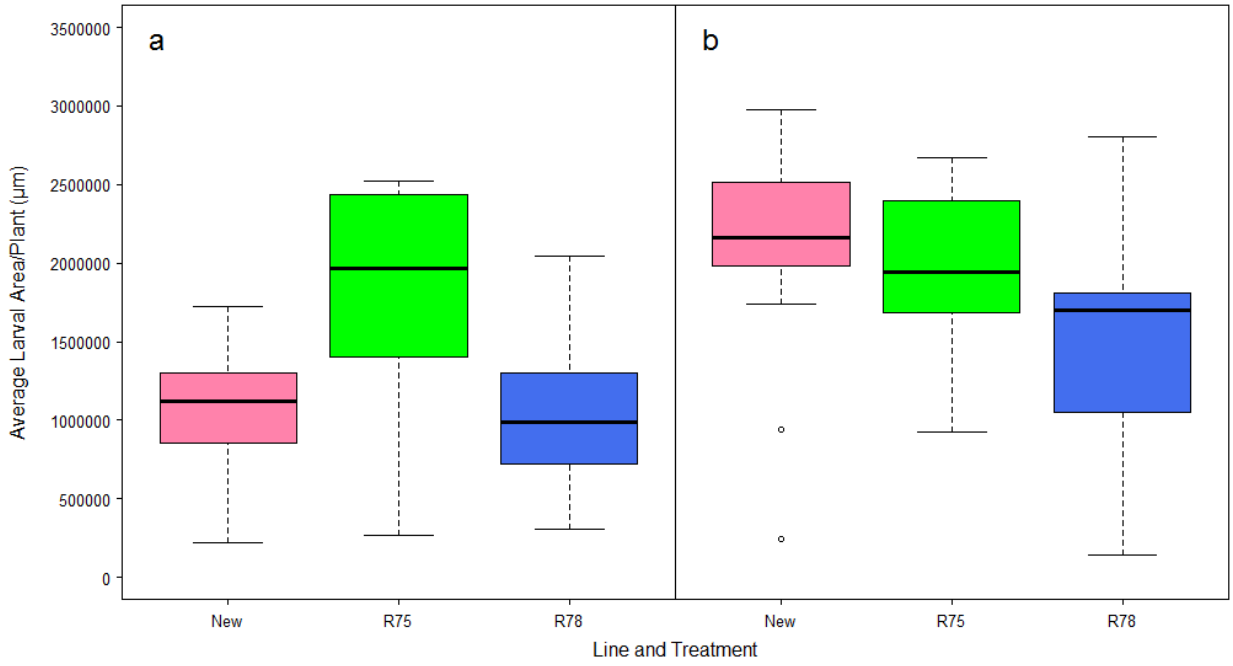

Figure 2.1 Relationship between average larval area, wheat line and treatment. a) Average larval area at 16 dpi. b) Average larval area at 32 dpi. Wheat lines: susceptible 'Newton' (New), susceptible Pioneer variety 25R75 (R75), and tolerant Pioneer variety 25R78 (R78) when infested (blue) and uninfested (pink). The red dot indicates sample mean, thick dark line indicates sample median, and first and third quartiles are indicated by the upper and lower edges. Minimum and maximum are indicated by the lower and upper range bar, while possible outliers in a sample are indicated by open circles.

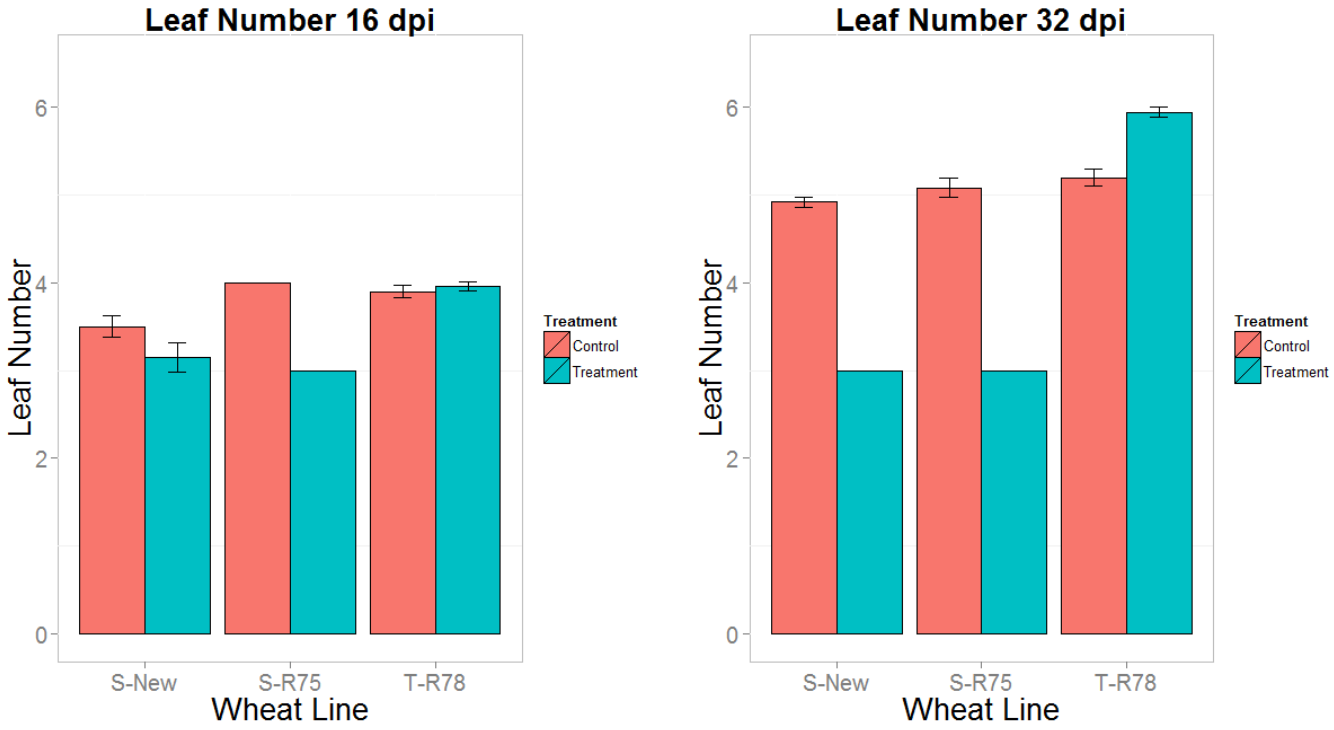

Figure 2.2 Relationship between leaf number, wheat line, and treatment. a) Leaf number at 16 dpi. b) Leaf number at 32 dpi. Wheat lines: susceptible 'Newton' (New), susceptible Pioneer variety 25R75 (R75), and tolerant Pioneer variety 25R78 (R78) when infested (blue) and uninfested (pink).

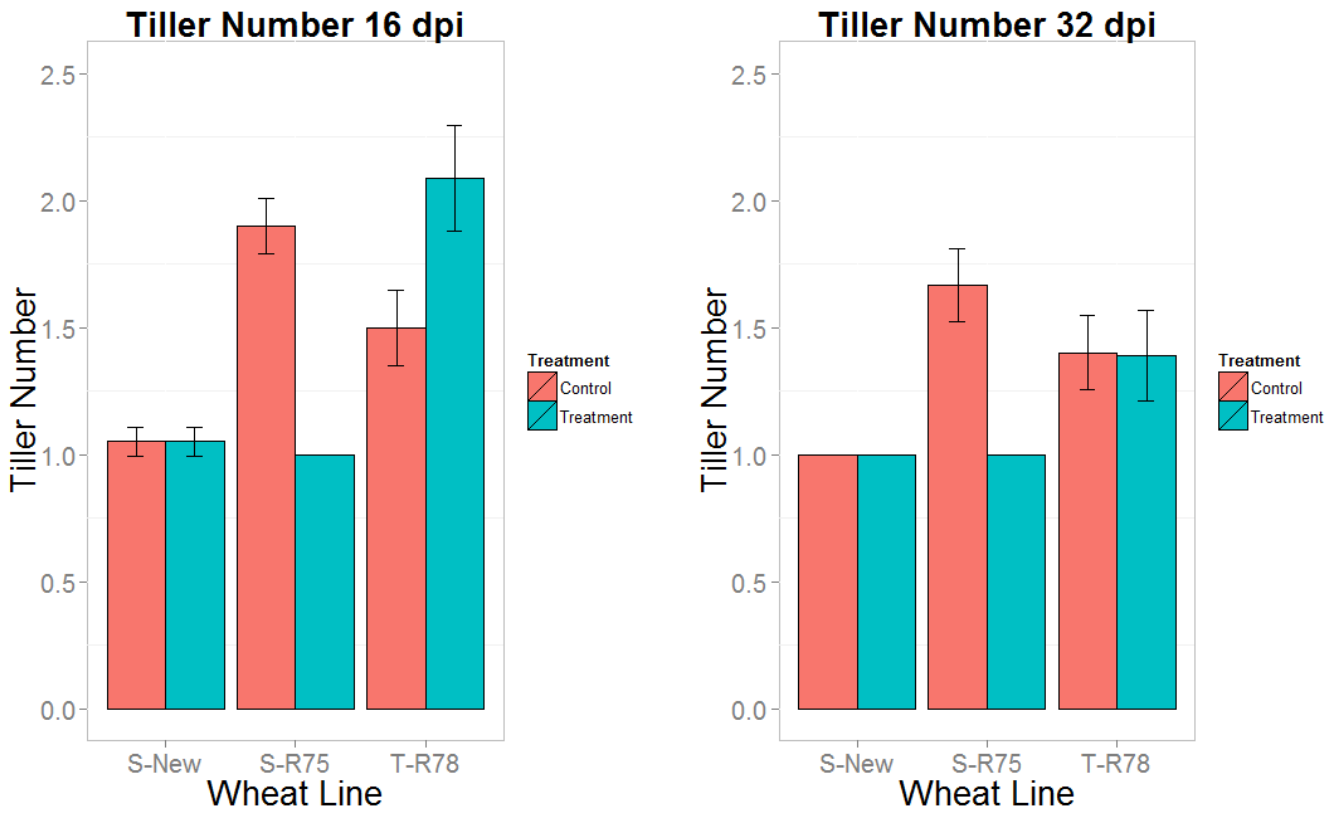

Figure 2.3 Relationship between tiller number, wheat line, and treatment. a) Tiller number at 16 dpi. b) Tiller number at 32 dpi. Wheat lines: susceptible 'Newton' (New), susceptible Pioneer variety 25R75 (R75), and tolerant Pioneer variety 25R78 (R78) when infested (blue) and uninfested (pink).

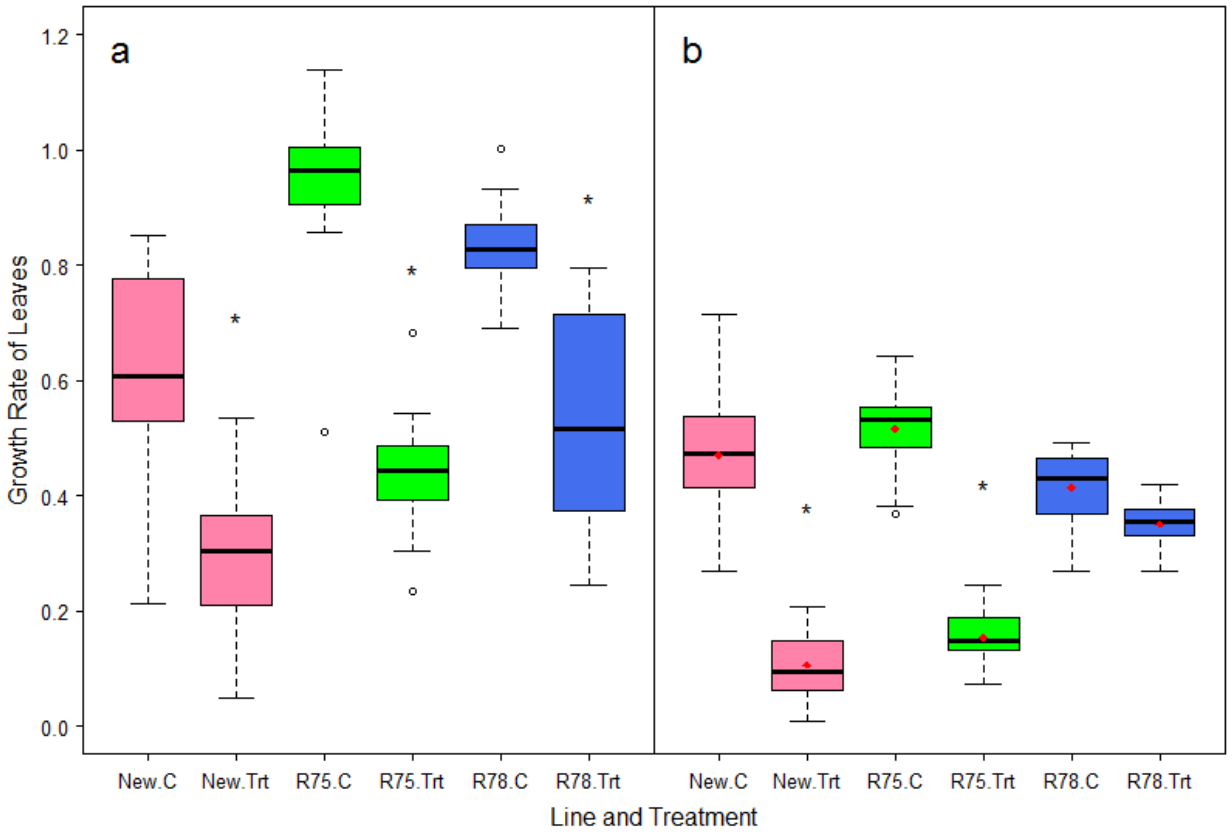

Figure 2.4 Relationship between leaf growth rate, wheat line, and treatment. a) Leaf growth rate at 16 dpi. b) Leaf growth rate at 32 dpi. Wheat lines: susceptible 'Newton' (New), susceptible Pioneer variety 25R75 (R75), and tolerant Pioneer variety 25R78 (R78) when infested (Trt) and uninfested (C). Treatments where leaf growth rate means differ significantly ($P < 0.05$) from their corresponding controls are indicated by a black asterisk, red dot indicates sample mean, thick dark line indicates sample median, and first and third quartiles are indicated by the upper and lower edges. Minimum and maximum are indicated by the lower and upper range bar, while possible outliers in a sample are indicated by open circles.

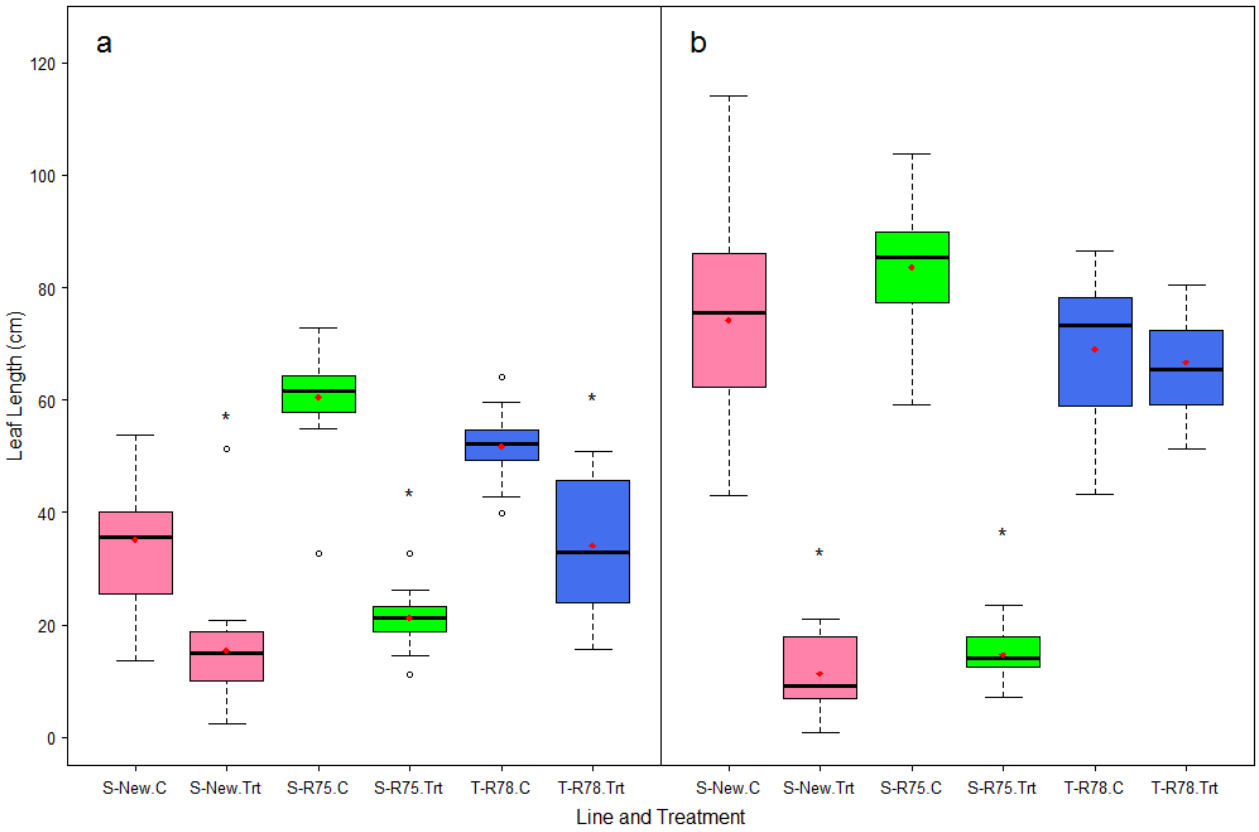

Figure 2.5 Relationship between total leaf length, wheat line and treatment. a) Change in the total leaf length at 16 dpi. b) Change in the total leaf length at 32 dpi. Wheat lines: susceptible 'Newton' (New), susceptible Pioneer variety 25R75 (R75), and tolerant Pioneer variety 25R78 (R78) when infested (Trt) and uninfested (C). Treatments where total leaf length means differ significantly ($P < 0.05$) from their corresponding controls are indicated by a black asterisk. The red dot indicates sample mean, thick dark line indicates sample median, and first and third quartiles are indicated by the upper and lower edges. Minimum and maximum are indicated by the lower and upper range bar, while possible outliers in a sample are indicated by open circles.

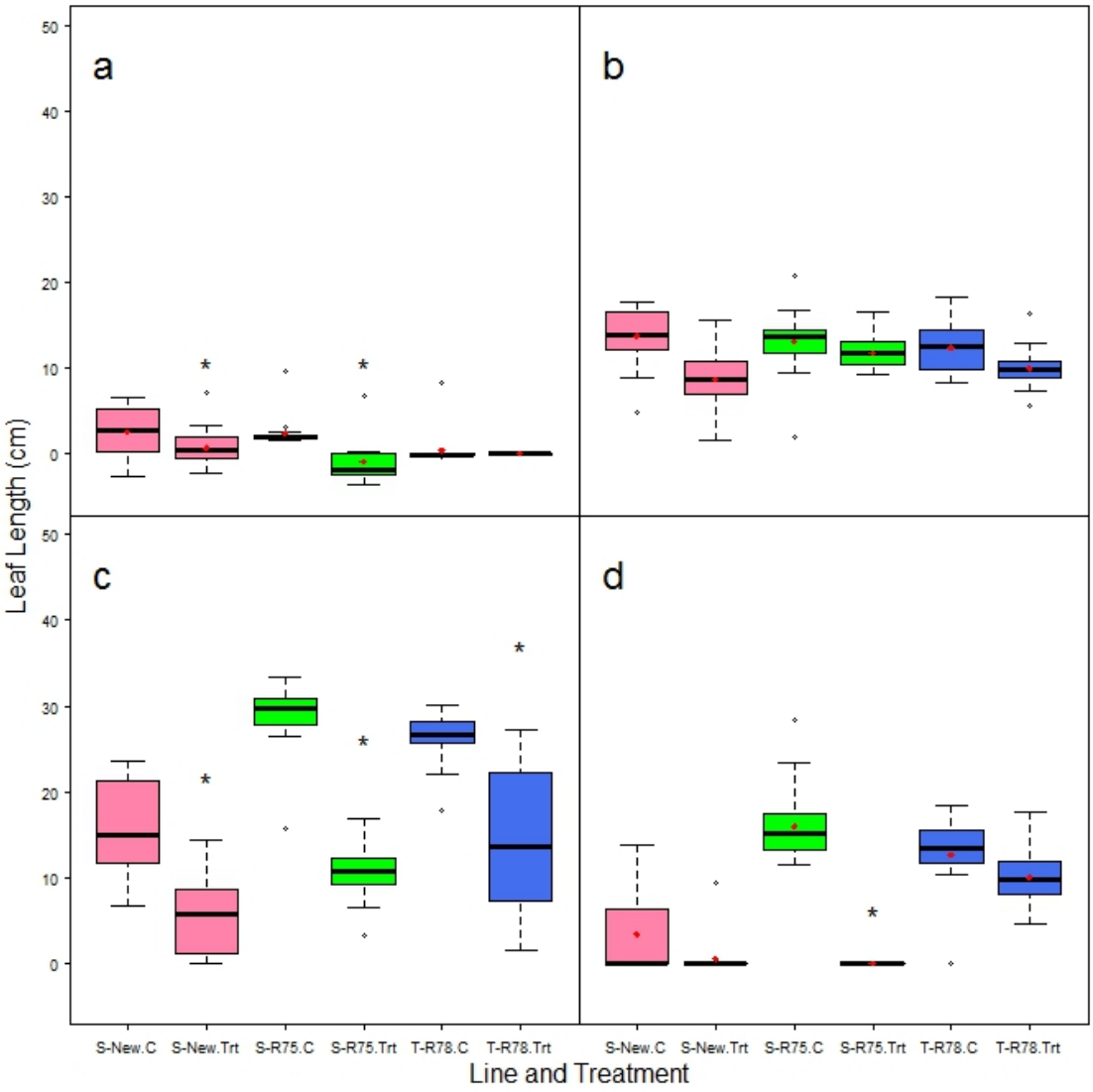

Figure 2.6 Relationship between individual leaf length changes, wheat line and treatment. a) Change in first leaf length at 16 dpi. b) Change in second leaf length at 16 dpi. c) Change in third leaf length at 16 dpi. d) Change in fourth leaf length at 16 dpi. Wheat lines: susceptible 'Newton' (New), susceptible Pioneer variety 25R75 (R75), and tolerant Pioneer variety 25R78 (R78) when infested (Trt) and uninfested (C). Treatments where individual leaf length means differ significantly ($P < 0.05$) from their corresponding controls are indicated by a black asterisk, red dot indicates sample mean, thick dark line indicates sample median, and first and third quartiles are indicated by the upper and lower edges. Minimum and maximum are indicated by the lower and upper range bar, while possible outliers in a sample are indicated by open circles.

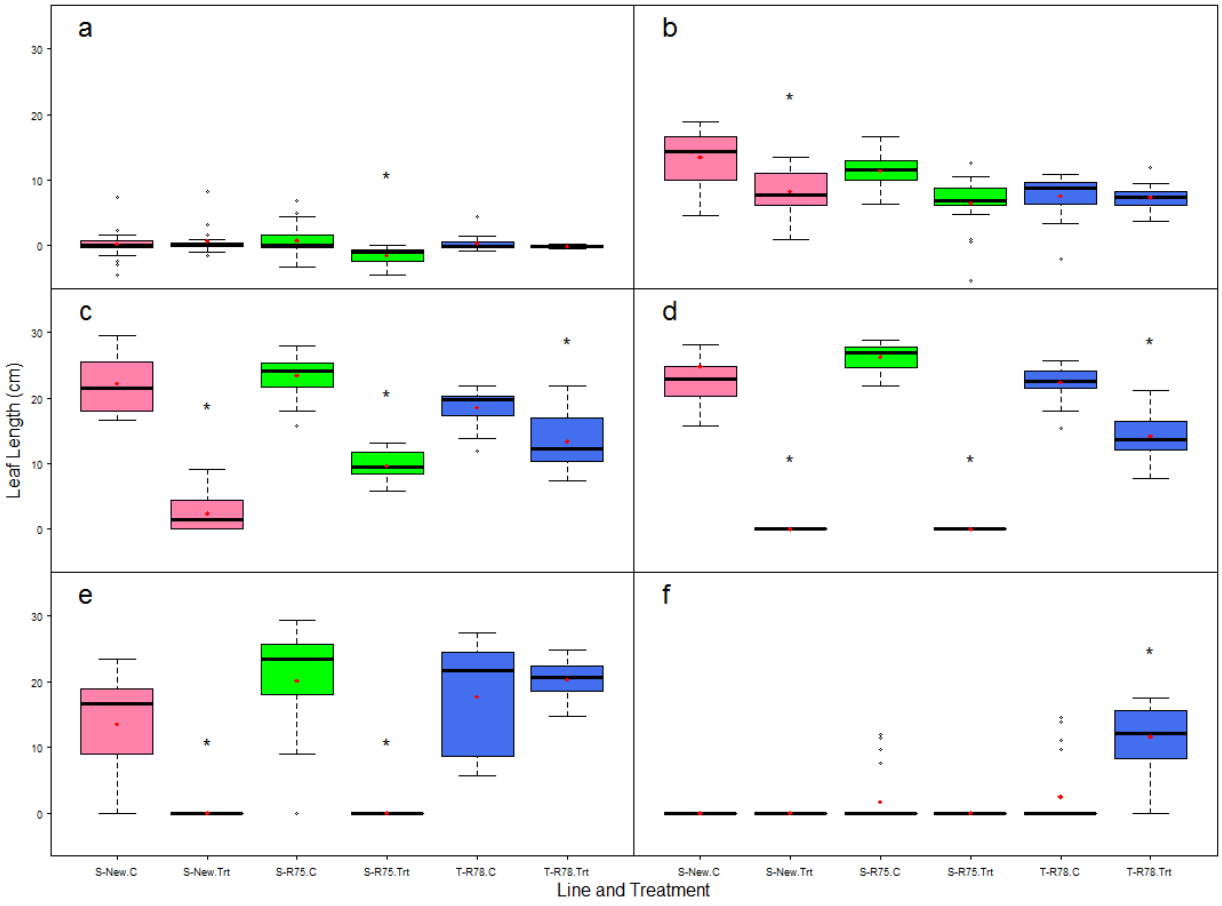

Figure 2.7 Relationship between the individual leaf length changes, wheat line and treatment. a) Change in first leaf length at 32 dpi. b) Change in second leaf length at 32 dpi. c) Change in third leaf length at 32 dpi. d) Change in fourth leaf length at 32 dpi. e) Change in fifth leaf length at 32 dpi. f) Change in sixth leaf length at 32 dpi. Wheat lines: susceptible 'Newton' (New), susceptible Pioneer variety 25R75 (R75), and tolerant Pioneer variety 25R78 (R78) when infested (Trt) and uninfested (C). Treatments where individual leaf length means differ significantly ($P < 0.05$) from their corresponding controls are indicated by a black asterisk, red dot indicates sample mean, thick dark line indicates sample median, and first and third quartiles are indicated by the upper and lower edges. Minimum and maximum are indicated by the lower and upper range bar, while possible outliers in a sample are indicated by open circles.

CHAPTER 3. FURTHER EXPERIMENTATION ON PLANT GROWTH EFFECTS AND LARVAL GROWTH EFFECTS

3.1 Abstract

The effects of Hessian fly infestation on the putative tolerant wheat line Pioneer variety 25R78 were previously analyzed. This was done by investigating leaf and tiller number as well as leaf growth rates and total leaf length change at two time intervals: 16 and 32 dpi. The overall results showed no permanent growth effects on tolerant wheat. To further study the effect of tolerance in Pioneer variety 25R78 on Hessian fly larvae, we used a time interval of 20 dpi and three wheat lines: Pioneer variety 25R78, the resistant 'Iris' variety, susceptible Pioneer variety 25R75, and susceptible variety 'Newton'. Both plant and larval effects were studied. For the plant effects, the measurements of leaf and tiller number, leaf growth rate, total leaf length change, and individual leaf length change were studied. For the larval effects, larvae size and number were studied.

New measurements included the number of visible larvae and their position on the plant in reference to the soil and first ligule. These measurements analyzed the effect tolerant plants have on larvae and their position if the plant continues to grow and not stunt. Results showed similar effects on the plant as those seen in the 32-day set in the first study. No permanent growth effects were visible since there were no significant

differences in leaf and tiller number as well as leaf length in infested tolerant plants compared to uninfested plants. However, leaf growth rate was significantly smaller in infested tolerant plants compared to uninfested plants, but significantly larger compared to the infested plants of the other lines. The data indicates that larval position on the plant is different on tolerant plants with significant differences in larval position occurring for infested tolerant plants compared to the other three lines.

3.2 Introduction

Hessian flies, or *Mayetiola destructor*, are some of the most destructive invasive pests of wheat worldwide. One of the major control methods is the use of *R*, or resistance, genes in wheat. These genes act in a gene-for-gene interaction with *Avirulence* (*AVR*) genes in the fly (Hatchett and Gallun, 1970; Gallun, 1977; Stuart et al., 2008). Flor (1955) proposed for that flax-rust interactions consisted of the presence of a corresponding parasite *AVR* gene for every plant *R* gene. This interaction also occurs between Hessian flies and wheat (Subramanyam et al., 2005). Compatible interactions, or those between virulent larvae and susceptible plants, occur when larvae successfully form a feeding site (Byers and Gallun, 1972). In these interactions, larvae can stunt plants, reduce yield, or kill plants (Buntin, 1999). Incompatible interactions occur when effectors released by the fly larvae into the plant trigger defense responses in the plant, called effector-triggered immunity (Williams et al., 2002). The larvae fail to form a successful feeding site and die within 3–5 days of the initial attack, without any growth (Ratcliffe and Hatchett, 1997; Agrios, 1997; Harris et al., 2006).

The disadvantage of resistance genes is the selection pressure exerted on surviving flies leads to the increased frequency of new virulent biotypes capable of overcoming *R* genes. An increased frequency of the virulent biotypes can occur any time after *R* gene deployment, sometimes occurring immediately after the release of the line or within 5–10 years (Ratcliffe and Hatchett, 1997; Ratcliffe et al., 2000; Cambron et al., 2010).

One possible solution would be to combine the ability to survive initial infestation and the absence of selection pressures on fly populations. This might be possible with tolerance in wheat to Hessian fly damage. Tolerance, in general, is the ability for a plant to recover, grow, and potentially reproduce despite pest attack, without placing selection pressures on the pest populations (Reese et al., 1994; Strauss and Agrawal, 1999). These plants tolerate damage, instead of harming or killing the pests (Painter, 1951).

In the previous study, growth effects from infestation with Hessian fly were studied in the putative tolerant Pioneer variety 25R78 were analyzed by measuring growth effects. The overall results showed no permanent growth effects on tolerant wheat at a young growth stage at 16 and 32 dpi. The absence of growth effects at these time intervals indicates the possibility for Pioneer variety 25R78 to tolerate larval feeding, reducing or preventing damage. The current study focuses on a combination of plant growth effects and how these affect the position of the larvae on the plant. In a compatible interaction, larvae can stunt a wheat seedling by stunting the third leaf and preventing further leaf production and growth (Harris et al., 1993). It was hypothesized that the recovery and continued growth of the tolerant plants would push the larvae out of the leaf sheath away from their feeding site. This could expose the larvae to adverse

environmental conditions such as desiccation, drowning, predation, or parasitism. This would then reduce fly populations without using a *R* gene to kill the larvae through effector-triggered immunity.

A few improvements were made for the current study. In this study, each plant was grown in a separate container. The container was a square, plastic pot. The first study included 10 plants grown in one pot. These pots were round plastic containers (10.16 cm. x 10.16 cm.). Another difference is the use of different growth chambers. The first study was done in a Percival growth chamber and the second study was performed in a Revco Honeywell growth chamber. The last difference was the day of the final measurement. The first study consisted of two sets of plants: one set measured at 16 dpi and one set measured at 32 dpi. The second study consisted of one set measured at 20 dpi. However, the method of planting and watering was the same. The timing between planting and infesting was the same as well. The temperature and humidity in the greenhouse and growth chamber were identical.

3.3 Materials and Methods

3.3.1 Plant and Insect Preparation

Four wheat lines were used: tolerant Pioneer brand variety 25R78, susceptible Pioneer brand variety 25R75, susceptible variety ‘Newton’, and resistant variety ‘Iris’. Each line had two sets: uninfested control and infested plants. The planting was staggered across subsequent days with Pioneer variety 25R78, Pioneer variety 25R75, ‘Newton’, and ‘Iris’ planted in that order. Each wheat line was planted on different days under identical environmental conditions due to logistical constraints. In a greenhouse (19°C ±

5°C), pots (3.10 cm. x 3.10 cm. x 2.33 cm., Traditional Inserts, and 18 pots/packs per insert) were filled with soil to within 2.54 cm of the rim. Water was added and the soil was mixed until no dry soil remained. After the pots drained, one seed was planted for each pot and gently pressed into the soil. Twenty seeds were planted for each line and set. More soil was mixed with water and 1 cm of this soil was added to each pot over the seed. Each pot was watered twice with only water to the rim. Then, they were watered with a solution of 2,300 mL water and 5.48 g of fertilizer (Scotts General Purpose, 20:20:20). This solution was made twice and each pot was watered with 200 mL twice after planting for a total of 400 mL. The plants remained in the greenhouse for 11 days after planting with watering as needed.

Hessian fly biotype E (avirulent/incompatible with wheat line *H3* 'Monon') was maintained by Sue Cambron at the USDA-ARS Crop Production and Pest Control Research Unit (West Lafayette, IN), according to the procedure described by Foster et al. (1988). While maintained, they were kept in 4°C cold storage with fluorescent lights. When ready to be used, wheat material with puparia was removed and placed in a clear plastic box (26 cm. by 39 cm.), moistened with a water-filled spray bottle, and kept under fluorescent lights at 18°C for 11 days until infestation.

3.3.2 Plant Infestation

On the 11th day, the pots were taken from the greenhouse into the lab at 18°C with 80% (± 5%) humidity. Each plant, including controls, were measured. Measurements included the distance from stem and soil for each leaf and ligule, leaf number, and tiller number. In order to keep plant measurements consistent, wooden stakes were used to

record treatment, assigned plant number (1–26 for each line and treatment), date, and wheat line. Each plant, including the controls, were covered with a clear, plastic 16 oz. cup (3 cm. diameter hole with mesh on top and 3–4 cm. hole on side with a foam plug) (Fig. D.1). The plants were at the 2–3 leaf stage (Zadoks Scale = 12 or 13; Zadoks et al., 1974). Measurements included the distance from stem and soil for each leaf and ligule.

Each plant, including the controls, were covered with plastic covers (16.9 oz. Meijer brand water bottles) with 2.54 cm of the bottom removed, a hole (2.54 diameter) cut on the side, and mesh hot-glued to the top (with the lid removed). One mated female fly was placed with an aspirator under each cover for infested plants and a styrofoam plug was placed in the hole. Flies remained for 6 hours and then were removed. Any plants in the treatment group that had no eggs or larvae present were removed from the analysis. The pots were kept inside under a fluorescent light at 18°C for 5 days. On the 5th day, the pots were placed in the growth chamber at 16°C (± 2°C) with a photoperiod of 16:8 (day:night) for 15 days (12d:12n). Each pot was watered with 200 mL tap water 4–5 days per week. Throughout that period, plants were observed and, if larvae were visible, the number, size, and distance of larvae from soil was noted and measured. After 20 days, post infestation, plants were measured with the same measurements as above. Plants were destroyed to count larvae and measure larva surface area.

3.3.3 Measurements Taken

Measurements were made immediately before infestation and 20 days after infestation. The measurements recorded included the following: length of leaves from

both the soil and stem, number of larvae found outside plant, total number of larvae, and larvae area. A leaf was measured if the leaf lamina was visible outside or above the whorl (Anderson and Harris, 2006; Anderson and Harris, 2008). Change in leaf length was calculated by subtracting the initial leaf measurement from the final leaf measurement and was an indicator of growth and leaf surface area. This was calculated for each leaf and the cumulative set of leaves for each plant and was used to calculate the change in total leaf length measurement. Using a clear ruler pressed into the soil, the leaf height was measured from the tip of the leaf to the soil. Leaf height to stem was also measured from the leaf tip to the first ligule. Measurements were rounded down to the nearest millimeter. Average leaf length was calculated by dividing the total leaf length change by the number of leaves per plant. Individual and total leaf lengths were measured, according to the methods of Anderson and Harris (2006).

Growth rate of the leaves at the stem was measured in several steps. First, each leaf was measured on the day of infestation and 20 dpi. The leaf was measured from the tip to the collar of the first ligule to control for movement of soil over time due to watering. The change in height was calculated by subtracting the initial measurement for each leaf from the final measurement. Then, this value was divided by 20 to calculate growth rate. The growth rates of all the leaves of a given plant were summed together and divided by the total number of leaves. This resulted in the overall average growth rate of leaves per plant.

Visible larvae were observed and counted from time of infestation to the final day of measurement. These larvae were visible from outside the leaf sheath, either on the leaves, stem, or soil, without destroying the plants. Analysis for visible larvae only

included plants with visible larvae. The distance from the stem was taken in relation to the first ligule. The distances that were measured below the first ligule were negative values with the first ligule counting as 0 cm. The distances measured above the first ligule were positive values with the first ligule counting as 0 cm. Distances of visible larvae from the soil was measured from the soil surface to the location of the visible larvae.

The larval counts were destructively taken after pulling the leaf sheath away from the plant. Larvae were removed from the leaf sheath, placed a clear glass slides, and analyzed under an Olympus SZX16 stereomicroscope with an attached Olympus dp210 camera. Larvae pictures were taken using the Olympus cellSens software and larvae area was measured using the "polygon" tool on cellSens. Larvae were classified as "living" if they were white with no red pigment while "dead" larvae were those that had not grown and showed red pigment (Zhang et al., 2011). Average area was calculated for each plant by summing the area of each larva and dividing the sum by the total number of larvae for that plant.

3.3.4 Statistical Analysis

Statistical analyses were performed in R (R Core Team, 2014). The design was a two-way factorial study with the following levels: wheat line and treatment (i.e. infested and uninfested). Wheat line had four levels: 'Newton', 'Iris', Pioneer variety 25R75, and Pioneer variety 25R78. Treatment had two levels: infested and uninfested. Sample sizes for infested plants indicate sample sizes for only plants that were successfully infested. Also, any pots without germination were removed. The sample sizes for the different

levels were the following: infested ‘Iris’ (n = 7), uninfested ‘Iris’ (n = 15), infested ‘Newton’ (n = 10), uninfested ‘Newton’ (n = 10), infested Pioneer variety 25R78 (n = 9), uninfested Pioneer variety 25R78 (n = 16), infested Pioneer variety 25R75 (n = 15), and uninfested Pioneer variety 25R75 (n = 17). A statistical significance threshold of $\alpha = 0.05$ was used.

Change in total and individual leaf length, distance of visible larvae from the stem and soil, leaf growth rate, and larval area were continuous variables with a normal distribution, while leaf, larvae, and tiller numbers were integer ratio scale variables. Each of these variables was analyzed using a two-way ANOVA. The model was made using the *aov* function of package in R (R Core Team, 2014).

3.4 Results

3.4.1 Visible Larvae

The wheat line appeared to have a significant impact on the number of visible larvae ($F = 10.52$, $df = 3$, $P = < 0.001$) (Table 3.4). The sum of squares was 288.3 and the mean of squares was 96.11. Infested tolerant plants had significantly more visible larvae than ‘Iris’, ‘Newton’, and Pioneer variety 25R75 ($P = < 0.001$ for all varieties) (Table 3.5). On the other hand, ‘Iris’ showed no significant differences from ‘Newton’ or Pioneer variety 25R75 ($P = 0.869$ and $P = 0.993$, respectively). Also, there was no significant difference between ‘Newton’ and Pioneer variety 25R75 ($P = 0.917$). ‘Iris’ did not have any visible larvae. The larvae on the ‘Iris’ plants may have died, preventing observation of the larvae outside the plant.

3.4.2 Distance of Visible Larvae from Soil and Stem

The distance of visible larvae from the stem measured the position of the larvae visible on the stem in relation to the first ligule. Wheat line had a significant effect on the position of visible larvae ($F = 20.84$, $df = 2$, $P = < 0.001$) (Table 3.3). The sum of squares was 43.71 and the mean of squares was 21.85. Tolerant plants had significantly greater distances on the stem compared to ‘Newton’, but not Pioneer variety 25R75 ($P = < 0.001$ and $P = 0.096$, respectively). There was no significant difference between Pioneer variety 25R75 and ‘Newton ($P = 0.266$) (Table 3.5; Fig. 3.3). ‘Iris’ was excluded from the analysis due to the lack of visible larvae.

The distance of visible larvae from the soil measured the position of the larvae visible on the stem (prior to plant destruction) compared to the soil. This allowed an estimate of how far the larvae were from the base of the plant. Wheat line had a significant effect on the position of visible larvae ($F = 18.79$, $df = 2$, $P = < 0.001$) (Table 3.4; Fig. 3.4). The sum of squares was 18.72 and the mean of squares was 9.361. Tolerant plants had significantly greater distances up the stem compared to Pioneer variety 25R75 and 'Newton' ($P = 0.009$ and $P = < 0.001$, respectively). There was no significant difference between Pioneer variety 25R75 and 'Newton ($P = 0.933$) (Table 3.5; Fig. 3.4). 'Iris' did not have any visible larvae and was not included in this analysis.

3.4.3 Leaf Number

Wheat line (F-value = 10.67, $P = < 0.001$), treatment (F-value = 49.75, $P = < 0.001$), and the interaction of the two (F-value = 21.98, $P = < 0.001$) appeared to have a significant effect on leaf number (Table 3.2). There was no significant difference between infested and uninfested plants for Pioneer variety 25R78 or 'Iris' ($P = 0.423$ and $P = 0.999$, respectively). Infested 'Newton' and Pioneer variety 25R75 showed significantly fewer leaves than their corresponding control plants ($P = < 0.001$ for both varieties) (Table 3.1; Fig. 3.1). Infested tolerant plants showed significantly more leaves than Pioneer variety 25R75 and 'Newton' ($P = < 0.001$ for both varieties). There was no significant difference between infested tolerant plants or infested 'Iris' plants ($P = 0.762$).

3.4.4 Tiller Number

The treatment (F-value = 22.11, P = < 0.001), line (F-value = 6.210, P = < 0.001), and the interaction between the two (F-value = 25.49, P = < 0.001) appeared to have a significant impact on tiller number (Table 3.2). There was no significant difference between infested and uninfested plants for Pioneer variety 25R78 or 'Iris' (P = 0.408 and P = 0.111, respectively) (Table 3.3; Fig. 3.2). Infested 'Newton' and Pioneer variety 25R75 plants showed significantly fewer tillers than their corresponding control plants (P = < 0.001 for both varieties). Infested tolerant plants showed significantly more tillers than Pioneer variety 25R75 and 'Newton' (P = < 0.001 for both varieties). There was no significant difference between infested tolerant plants or infested 'Iris' plants (P = 0.985).

3.4.5 Change in Total Leaf Length

The treatment (F-value = 149.6, P = < 0.001), line (F-value = 9.683, P = < 0.001), and the interaction between the two (F-value = 19.51, P = < 0.001) appeared to have a significant impact on the change in total leaf length (Table 3.2). The change in total leaf length was not significantly different between infested and uninfested plants for Pioneer variety 25R78 or for 'Iris' (P = 0.4659 and P = 0.6562, respectively) (Table 3.3; Fig. 3.6). However, change in total leaf length for infested plants of Pioneer variety 25R75 and 'Newton' were significantly smaller than uninfested plants (P = < 0.001 for both varieties) (Table 3.3; Fig, 3.7a). Infested tolerant plants showed significantly greater total leaf length changes than infested plants of 'Newton' or Pioneer variety 25R75 (P = < 0.001 for both varieties).

3.4.6 Individual Leaf Length Changes

Infested Pioneer variety 25R78 plants had significantly smaller leaf lengths for the third and fourth leaf lengths (P = < 0.001 for both leaves) (Table 3.6). However, the infested Pioneer variety 25R78 plants showed no significant difference in leaf length for the other leaves compared to the control plants (Table 3.6). Pioneer variety 25R75 showed significant differences in leaf length changes for each leaf except the ninth and tenth leaves (Table 3.6). Infested 'Newton' showed significantly smaller first, second, eighth, and ninth leaves (Table 3.6). Infested 'Iris' plants showed no significant difference in the length of any leaves. For the third leaf, infested tolerant plants showed no significant difference in length compared to infested Pioneer variety 25R75 or 'Iris' (P = 0.970 and P = 0.999, respectively). They did have significantly longer leaves compared to the third leaves of infested 'Newton' plants (P = 0.039). For the fourth leaf, infested tolerant plants showed no significant difference in length compared to infested 'Iris' plants (P = 0.980). They did have significantly longer leaves compared to the fourth leaves of infested Newton and Pioneer variety 25R75 (P = < 0.001 for both varieties).

3.5 Discussion

In this study, Pioneer variety 25R78 was analyzed in respect to tolerance to Hessian fly. The Hessian fly feeds as a larva at a feeding site within the leaf sheath. In compatible interactions, larvae feed successfully and develop normally. In incompatible interactions, the larvae fail to establish a feeding site, leading to death. However, the first study demonstrated the ability for tolerance in wheat to permit plants to survive and grow despite larval attack, without killing the larvae. Tolerance was further studied in this

study, as well as the effects on larvae. Results support the 32-day set results from the first study. Larvae had no negative effect on leaf or tiller number, total leaf length, or individual leaf length, except for the third and fourth leaves. Effects on larvae resulted in smaller larvae, more visible larvae, and greater distances of visible larvae from the soil and first ligule on tolerant plants. The results of each measurement provides support for the results of the first study as well as a better understanding of tolerance and effects on larvae.

Unlike in the first study in Chapter 2, this study showed no significant differences in larvae number between the tolerant line and any of the other lines. This lack of difference might be due to continual observation for larvae, preventing any larvae from falling off the plant unnoticed and recorded. However, there were differences in visible larvae. The only line to have any significant difference in the number of visible larvae was the tolerant line. The tolerant line had significantly more visible larvae than the resistant and two susceptible lines (Table 3.5).

The results indicate that larvae on tolerant plants do not always remain within the leaf sheath. This could be due to the recovery and growth of the plant, pushing the larvae out of the sheath. Regardless of the reason, the presence of visible larvae indicate that the larvae are no longer within the protective leaf sheath, potentially exposing them to harmful conditions, such as drowning, desiccation, or parasitism. These visible larvae might also be displaced from their feeding sites, possibly resulting in starvation, poor growth, and death.

In this study, only ‘Iris’ had dead red larvae. Despite the lack of larval death, there appeared to be a negative effect on growth of the larvae on the tolerant plants. This could

be considered antibiosis by the definition provided by Painter (1951), which takes into account any mechanism that affects a pest's growth, development, or survival. In this case, larvae are significantly smaller on tolerant plants, indicated some method of growth reduction. Reduction in larval size might be due to lectins or displacement from the leaf sheath as can be noted in the following Section 3.4.4. The absence of dead red larvae on the tolerant line potentially demonstrates that larval antibiosis was incomplete unlike the fatal antibiosis incurred by resistant plants where the majority of larvae are killed before they grow (Shukle et al., 1990). The absence of dead larvae can also allow tolerant plants to reduce selection pressures placed on fly populations.

The tolerant plants had visible larvae with positions farther up the stem in relation to the soil at a greater distance compared to the two susceptible lines and the resistant line. Tolerant plants also had larvae at significantly higher positions in relation to the first ligule than 'Newton' plants. Resistant 'Iris' did not have any visible larvae so there were no measurements to be made. It was difficult to distinguish whether larvae were visible on susceptible plants due to actual plant growth or due to splitting of the stem caused by the larvae.

The position of the larvae compared to the first ligule helped clarify some of this confusion. Although susceptible Pioneer 25R75 and tolerant plants had no significant difference in larval position in relation to the first ligule, tolerant plants were the only plants to have visible larvae above the first ligule (Appendix B). This indicates the presence of the larvae outside the leaf sheath. These results indicate that more larvae are present outside the leaf sheath of tolerant plants, potentially exposing them to adverse conditions. Their presence outside the leaf sheath could be caused by tolerant plant

growth recovery, pushing the larvae outside of the leaf sheath. Greater numbers of exposed larvae could lead to more larval death, potentially reducing fly populations without using a *R* gene.

Similar to the 16-day and 32-day sets in the first study, tolerant plants showed no negative growth effects on leaf or tiller number, while both susceptible lines in this study showed negative effects in the form of leaf and tiller loss ($P = < 0.001$ for both lines and measurements) (Table 3.1 and 3.3; Fig. 3.2). Similar to the tolerant line, resistant variety 'Iris' showed no difference in leaf or tiller number ($P = 1.000$ and $P = 0.111$, respectively). The absence of leaf or tiller loss for Pioneer variety 25R78 strongly supports the hypothesis that Pioneer variety 25R78 is indeed tolerant to Hessian flies. The results indicate infested tolerant plants do not have long-term effects in stunting or growth with growth comparable to uninfested tolerant plants and uninfested resistant 'Iris' plants.

Leaf loss prevention is an important aspect of plant growth and could lead to increased biomass and comparable yield to uninfested plants (Anderson et al., 2011). While infested tolerant plants showed no leaf loss, infested susceptible 'Newton' plants in other studies did show leaf loss. The susceptible 'Newton' plants have been reported to show severe growth deficits in main tiller/stem leaves with very few infested plants showing a fourth leaf, as well as the complete absence of a fifth leaf (Anderson and Harris, 2008). The absence of leaf loss in tolerant plants might be a result of compensation, also known as the degree of tolerance displayed by the plants (Strauss and Agrawal, 1999). Full compensation occurs when undamaged and damaged plants show the same fitness or growth (Strauss and Agrawal, 1999).

Similar to the results for the 32-day set in the first study, tolerant plants showed no negative growth effects from infestation on total leaf length (P = 0.466) (Table 3.3; Fig. 3.6.). The absence of growth effects for tolerant plants is consistent with the absence of growth effects for the resistant variety 'Iris' (P = 0.656). This absence indicates the ability for tolerant plants to overcome damage from larval feeding and grow competitively compared to uninfested plants. This absence of growth effects on total leaf length can help provide greater leaf length, potentially increasing the leaf area index and biomass, and leading to an increase in yield (Petcu, 2003).

The growth rate of leaves (GR) of the infested tolerant plants were significantly smaller than those of the uninfested plants (P = 0.002) (Appendix B; Table 3.3). However, infested tolerant plants showed no significant difference in GR compared to infested resistant 'Iris' plants. Even the resistant 'Iris' plants showed significantly smaller GR when infested. Although tolerant plants show negative effects on GR when infested, the same is true for resistant plants. These effects on GR for both tolerant and resistant plants might be due to a decrease in overall changes in leaf length since this makes up a component for calculating GR. This might occur due to stunting of just a few leaves.

No significant differences for tolerant plants in leaf number, tiller number, and CTL could potentially increase leaf biomass and yield. Biomass and leaf area index are positively correlated until the anthesis phase (Petcu, 2003). Also, yield and biomass of certain winter wheat lines are positively correlated while biomass and yield, number of ears/m^2, and number of seeds are positively correlated (Petcu, 2003).

Although there was initial stunting in the third and fourth leaf, the infested tolerant plants showed superior growth compared to their susceptible counterparts. For

example, the infested tolerant plants showed significantly greater changes in the length of the third leaf compared to that of infested ‘Newton’ plants (P = 0.039), but not infested ‘Iris’ (P = 0.999) or Pioneer variety 25R75 (P = 0.970). Infested tolerant plants showed significantly longer leaf lengths for the fourth leaf compared to Pioneer variety 25R75 (P = < 0.001) and ‘Newton’ (P = < 0.001), but not ‘Iris’ (P = 0.995).

This superior growth is evident in the difference in leaf number and leaf length. Anderson and Harris (2008) reported that susceptible ‘Newton’ showed significant larval effects on the growth of third, fourth, and fifth leaves, with the complete absence of a fifth leaf. This may demonstrate the ability of tolerant plants to recover and grow (Table 3.6). Additionally, the lack of growth effects on the other tolerant plant leaves, including those that emerge after larval feeding has begun, indicates that, although initial stunting occurred, tolerant plants are able to recover and grow (Table 3.6). This is reminiscent of the ability of resistant plants to recover in growth from stunting of third and fourth leaves by normal growth of later-emerging leaves. However, unlike resistant plants, the growth recovery of later-emerging leaves on tolerant plants occurs during larval feeding, instead of after larval death (Anderson and Harris, 2008).

Several resistant wheat lines have also shown initial stunting for the third and fourth leaves similar to that of the tolerant Pioneer variety 25R78. A previous study analyzed the growth effects of larval feeding for lines *H9*, *H13*, and *H6* using the sampling times of 36, 156, and 348 hours (Anderson and Harris, 2006). It was concluded that *R* genes cannot stop larvae from affecting the leaf growth zones and the effects on leaf growth can be systemic (Anderson and Harris, 2006). However, despite the stunted third and fourth leaves, the fifth and main tiller leaves showed quick growth due to

availability of resources within the plant, resulting in no permanent growth effects (Anderson and Harris, 2006). Unlike that study, this research consisted of two measurements: immediately before infestation and 20 days after infestation due to logistical constraints. Although this reduces the number of time points, it still allows for an analysis of final growth effects after 20 dpi. Continued leaf growth and production is important because grain yield can be determined by the maximum leaf area index as well as total water supply (Benbi, 1994).

On tolerant plants, larvae can feed successfully and stunt the leaves just beginning to emerge at initial attack (i.e. third and fourth leaves). However, the absence of stunting in every other leaf of tolerant plants demonstrates the ability to overcome stunting of the plant and prevent stunting of the leaves that emerge after initial infestation and feeding, similar to how resistant plants overcome stunting (Anderson and Harris, 2011). As discussed in Sections 3.4.4–3.4.6, Pioneer 25R78 plants tolerated larval feeding with no loss in leaf or tiller number, as well as a reduction or prevention of growth effects on total leaf length. These results indicate that Pioneer 25R78 plants demonstrate the tolerance defined by Strauss and Agrawal (1999) as the ability to recover and grow despite insect attack. This aspect is important for plant survival and yield.

3.6 Conclusion

The tolerant plant growth measurements, except for leaf growth rate and the leaf lengths for the third and fourth leaves, showed no negative growth effects from infestation. This confirmed the results from the first study with mutual prevention of leaf and tiller loss, reduction or prevention of loss in total leaf length, and overall recovery

from stunting in individual leaf length. This supports the conclusion that Pioneer variety 25R78 plants are able to tolerate and recover from Hessian fly feeding damage, possibly preventing future yield loss.

Tolerant plants also showed significantly more visible larvae compared to the other lines. These larvae were also found higher up the plant than the larvae on other lines. In fact, visible larvae on the tolerant plants were the only larvae to be found above the first ligule and above the leaf sheath. This indicates the possibility for larvae to be pushed out of the leaf sheath. This displacement might be due to the failure of the larvae to stunt the plants, leading to continued plant growth. The increased number of larvae outside the leaf sheath leads to greater larval exposure to adverse conditions, decreasing fly population numbers while preventing selection pressures on these populations.

3.7 References

Agrios, G.N. 1997. Plant diseases caused by Mollicutes: Phytoplasmas and Spiroplasmas, pp 457–470. *In*: Agrios, G.N. (Ed.), Plant Pathology, Academic Press, New York.

Anderson, K.G., M.O. Harris. 2006. Does *R* gene resistance allow wheat to prevent plant growth effects associated with Hessian Fly (Diptera : Cecidomyiidae) attack? Plant Resistance. 99(5): 1842–1853.

Anderson, K.G., M.O. Harris. 2008. Leaf growth signals the onset of effective resistance against Hessian fly larvae. Entomol. Exp. Appl. 128(1): 184–195.

Byers, R.A., R.L. Gallun. 1972. Ability of the Hessian fly to stunt winter wheat. 1. Effects of larval feeding on elongation of leaves. J. Econ. Entomol. 65: 955–958.

Cambron, S.E., G.D. Buntin, R. Weisz, J.D. Holland, K.L. Flanders, B.J. Schemerhorn, R.H. Shukle. 2010. Virulence in Hessian fly (Diptera: Cecidomyiidae) field collections from the southeastern United States to 21 resistance genes in wheat. J. Econ. Entomol. 103: 2229–2235.

Flor, H.H. 1955. Host-parasite interaction in flax rust-its genetics and other implications. Phytopathology. 45: 680–685.

Gallun, R.L. 1977. Genetic basis of Hessian fly epidemics. Ann. N.Y. Acad. Sci. 287: 222–229.

Harris, M.O., S. Rose, P. Malsch. 1993. The role of vision in the host plant-finding behavior of the Hessian fly. Physiol. Entomol. 18: 31–42.

Harris, M.O., T.P. Freeman, O. Rohfritsch, K.G. Anderson, S.A. Payne, J.A. Moore. 2006. Virulent Hessian fly (Diptera: Cecidomyiidae) larvae induce a nutritive tissue during compatible interactions with wheat. Ann. Entomol. Soc. Am. 99(2): 305–316.

Hatchett, J. H., R.L. Gallun. 1970. Genetics of the ability of the Hessian fly, *Mayetiola destructor*, to survive on wheat having different genes for resistance. Ann. Entomol. Soc. Am. 63: 1400–1407.

Painter, R.H. 1951. Insect Resistance in Crop Plants. Macmillan, New York, NY, USA.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. URL http://www.R-project.org/.

Ratcliffe, R.H., J.H. Hatchett. 1997. Biology and genetics of the Hessian fly and resistance in wheat, pp. 47–56. *In*: Bondarie, K. (Ed.), New Developments in Entomology, Research Signpost. Scientific Information Guild, Trivandrum, India.

Ratcliffe, R.H., S.E. Cambron, K.L. Flanders, M.A. Bosque-Peres, S.L. Clement, H.W. Ohm. 2000. Biotype composition of Hessian fly (Diptera: Cecidomyiidae) populations from the Southeastern, Midwestern, and Northwestern United States and virulence to resistant genes in wheat. J. Econ. Entomol. 93: 1319–1328.

Reese, J., J. Schwenke, P. Lamont, D. Zehr. 1994. Importance and Quantification of Plant Tolerance in Crop Pest Management Programs for Aphids: Greenbug Resistance in Sorghum. J. Agr. Entomol. 11: 255–270.

Strauss, S., A. Agrawal. 1999. The ecology and evolution of plant tolerance to herbivory. TREE. 14: 179–185.

Stuart, J.J., M.S. Chen, M. Harris. 2008. Hessian fly, pp 93-100. *In*: Hunter, W., C. Kole (Eds.), Genome Mapping and Genomics in Animals, vol. 1: Genome Mapping and Genomics in Arthropods. Springer, Berlin, Heidelberg, New York.

Subramanyam, S., N. Sardesai, D.P. Puthoff, J.M. Meyer, J.A. Nemacheck, M. Gonzalo, C. E. Williams. 2005. Expression of two wheat defense-response genes, Hfr-1 and *Wci-1*, under biotic and abiotic stresses. Plant Sci. 170: 90–103.

Williams, C.E., C.C. Collier, J.A. Nemacheck, L. Chengzhi, S.E. Cambron. 2002. A lectin-like wheat gene responds systemically to attempted feeding by avirulent first-instar Hessian fly larvae. J. Chem. Ecol. 28: 1411–1428.

Zadok, J. C., T.T. Chang, C.F. Konzak. 1974. A decimal code for the growth stages of cereals. Weed Res. 14: 415–421.

3.8 Tables and Figures

Table 3.1 Mean leaf number for each treatment of the different wheat lines

	Treatment	Mean (Standard Deviation)	p-value
'Newton	C‡	6.900 (1.101)	< 0.001***
	T§+	3.333 (1.000)	
Pioneer variety 25R75	C	8.471 (0.717)	< 0.001***
	T	4.133 (1.995)	
Pioneer variety 25R78	C	7.438 (1.788)	0.423
	T	8.750 (1.389)	
'Iris	C	7.333 (0.976)	0.999
	T	7.571 (2.149)	

* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$.
† dpi: days post infestation
‡ 'C' indicates control or uninfested plants.
§ 'T' indicates Hessian fly-infested plants.

Table 3.2 Results of a two-way ANOVA analysis for leaf number, tiller number, leaf growth rate, and total leaf length as determined by two-way ANOVA.

Measurement	Wheat Line	F-value	P value	Df	Sum. Sq.	Mean. Sq.
Leaf Number	Treatment	49.75	< 0.001***	1,89	103.6	103.6
	Wheat Line	10.67	< 0.001***	3,89	66.69	22.23
	Treatment: Wheat Line	21.98	< 0.001***	3,89	137.4	45.78
Tiller Number	Treatment	21.36	< 0.001***	1,89	7.990	7.989
	Wheat Line	5.000	0.003**	3,89	5.610	1.870
	Treatment: Wheat Line	24.22	< 0.001***	3,89	27.17	9.058
Leaf Growth Rate	Treatment	145.8	< 0.001***	1,89	1.371	1.371
	Wheat Line	7.825	< 0.001***	3,89	0.221	0.074
	Treatment: Wheat Line	6.065	< 0.001***	3,89	0.171	0.057
Change in Total Leaf Length	Treatment	149.60	< 0.001***	1,89	140.2	140.2
	Wheat Line	9.683	< 0.001***	3,89	27.22	9.07
	Treatment: Wheat Line	19.51	< 0.001***	3,89	54.84	18.28

* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$.

Table 3.3 Post-hoc Tukey analysis of tiller number, leaf growth rate, and total leaf length between treatments of each wheat line.

Measurement	Wheat Line	P value
Tiller Number	‘Newton’	< 0.001***
	25R75	< 0.001***
	25R78	0.408
	‘Iris’	0.111
Leaf Growth Rate	‘Newton’	< 0.001***
	25R75	< 0.001***
	25R78	0.002**
	‘Iris’	0.048*
Change in Total Leaf Length	‘Newton’	< 0.001***
	25R75	< 0.001***
	25R78	0.466
	‘Iris’	0.656

* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$.

Table 3.4 Results of a one-way ANOVA analysis for visible larvae number, average larval area, and distance of visible larvae from the soil and from the first ligule.

Measurement	Wheat Line	F-value	P value	df	Sum. Sq.	Mean. Sq.
Larvae Number	Wheat Line	3.181	0.036*	2,63	719.1	239.7
Visible Larvae Number	Wheat Line	10.52	< 0.001***	2,63	288.3	96.11
Larval Area	Wheat Line	18.75	< 0.001***	2,63	3.179×10^{13}	1.060×10^{13}
Distance of VL to 1st ligule	Wheat Line	20.84	< 0.001***	2,63	43.71	21.85
Distance of VL to Soil	Wheat Line	18.79	< 0.001***	2,63	18.72	9.361

* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$.

Table 3.5 Post-hoc Tukey analysis on larvae number, visible larvae number, average larval area, and distance of visible larvae from the soil and from the first ligule.

Measurement	Wheat Line	P value
Larvae Number	25R78-'Newton'	0.440
	25R78-25R75	0.211
	25R78-'Iris'	0.903
Visible Larvae Number	25R78-'Newton'	0.001***
	25R78-25R75	< 0.001***
	25R78-'Iris'	< 0.001***
Larval Area	25R78-'Newton'	< 0.001***
	25R78-25R75	< 0.001***
	25R75-'Newton'	0.781
Distance of VL to 1st ligule	25R78-'Newton'	< 0.001***
	25R78-25R75	0.096
	25R75-'Newton'	0.266
Distance of VL to Soil	25R78-'Newton'	< 0.001***
	25R78-25R75	0.009
	25R75-'Newton'	0.933

* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$.

Table 3.6 Post-hoc Tukey analysis on individual leaf length.

Wheat Line	First	Second	Third	Fourth	Fifth	Sixth
25R78	0.999	0.999	< 0.001***	< 0.001***	0.997	0.710
25R75	0.036*	< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001***
'Newton'	0.870	0.565	< 0.001***	< 0.001***	< 0.001***	< 0.001***
'Iris'	1.000	0.564	0.115	0.075	0.629	0.999

Wheat Line	Seventh	Eighth	Ninth	Tenth	Eleventh
25R78	0.999	1.000	0.996	0.912	0.082
25R75	< 0.001***	< 0.001***	0.195	0.948	†
'Newton'	0.030**	0.999	0.999		
'Iris'	1.000	0.974	0.999	0.801	

* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$.
†: indicates that the leaf was nonexistent or too small to measure at that sampling time.

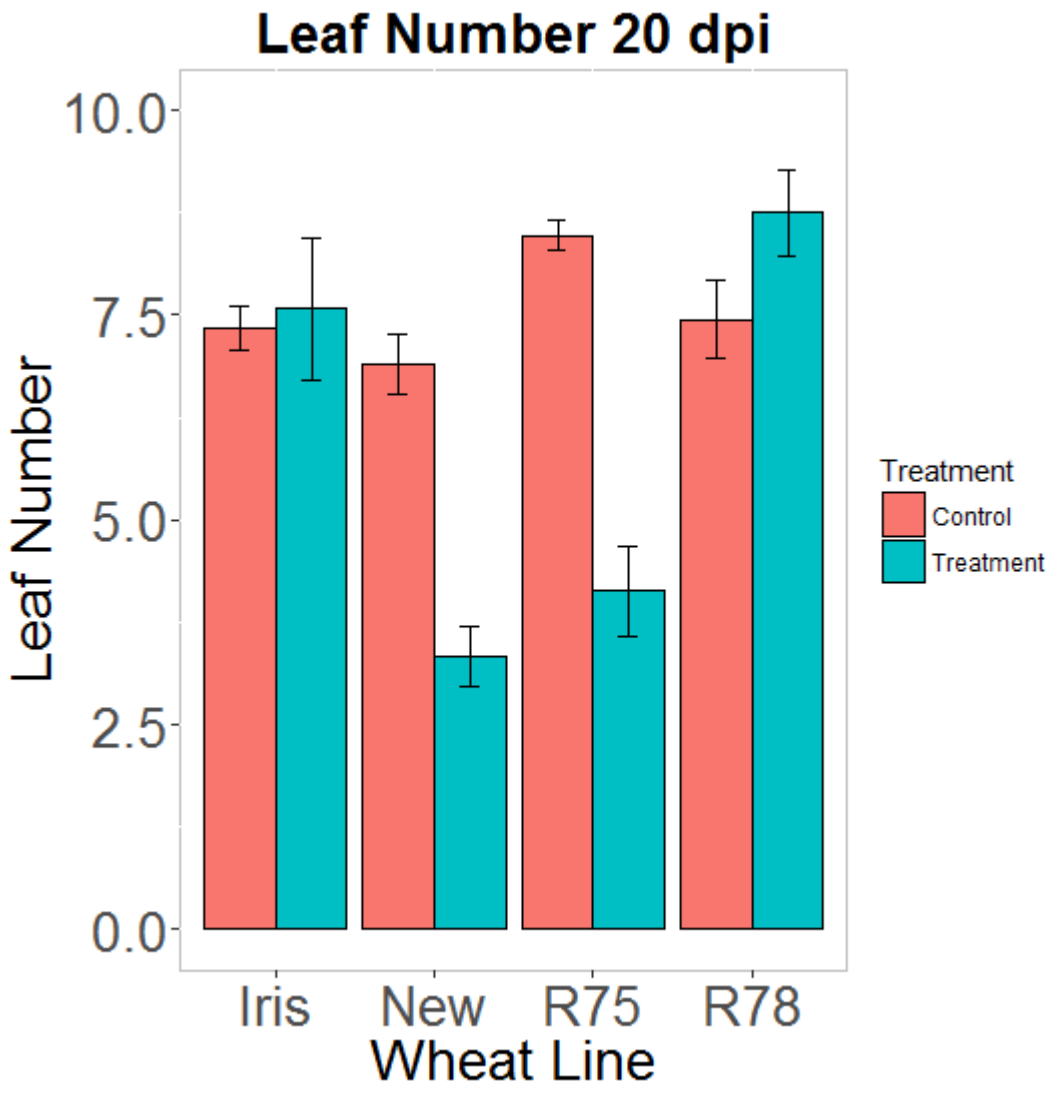

Figure 3.1 Relationship between leaf number, wheat line and treatment. Wheat lines: resistant 'Iris (Iris), susceptible 'Newton' (New), susceptible Pioneer variety 25R75 (R75), and tolerant Pioneer variety 25R78 (R78) when infested (blue) and uninfested (pink). Error bars represent standard errors of each sample.

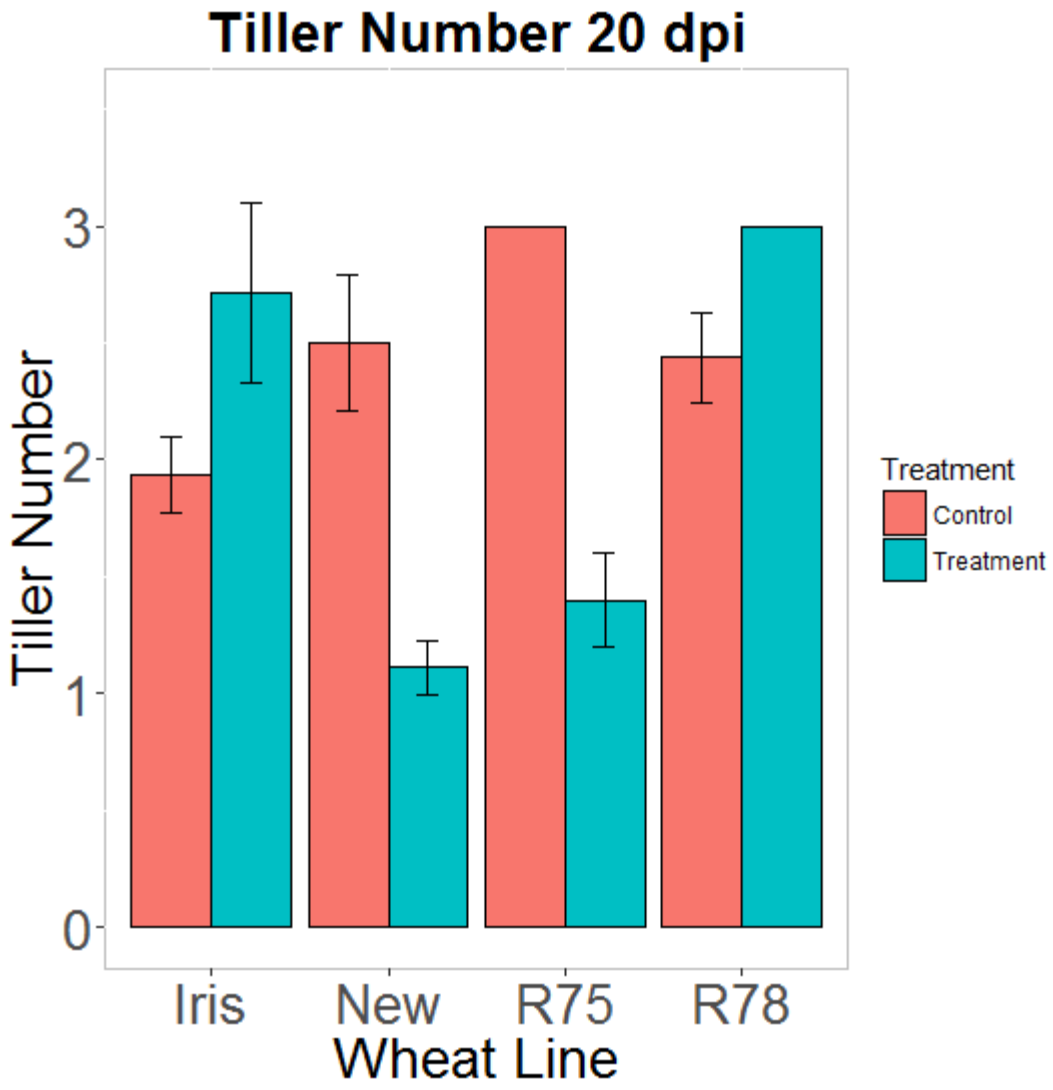

Figure 3.2 Relationship between tiller number, wheat line and treatment. Wheat lines: resistant 'Iris (Iris), susceptible 'Newton' (New), susceptible Pioneer variety 25R75 (R75), and tolerant Pioneer variety 25R78 (R78) when infested (blue) and uninfested (pink). Error bars represent standard errors of each sample. Lack of bars signifies lack of variation for that treatment set.

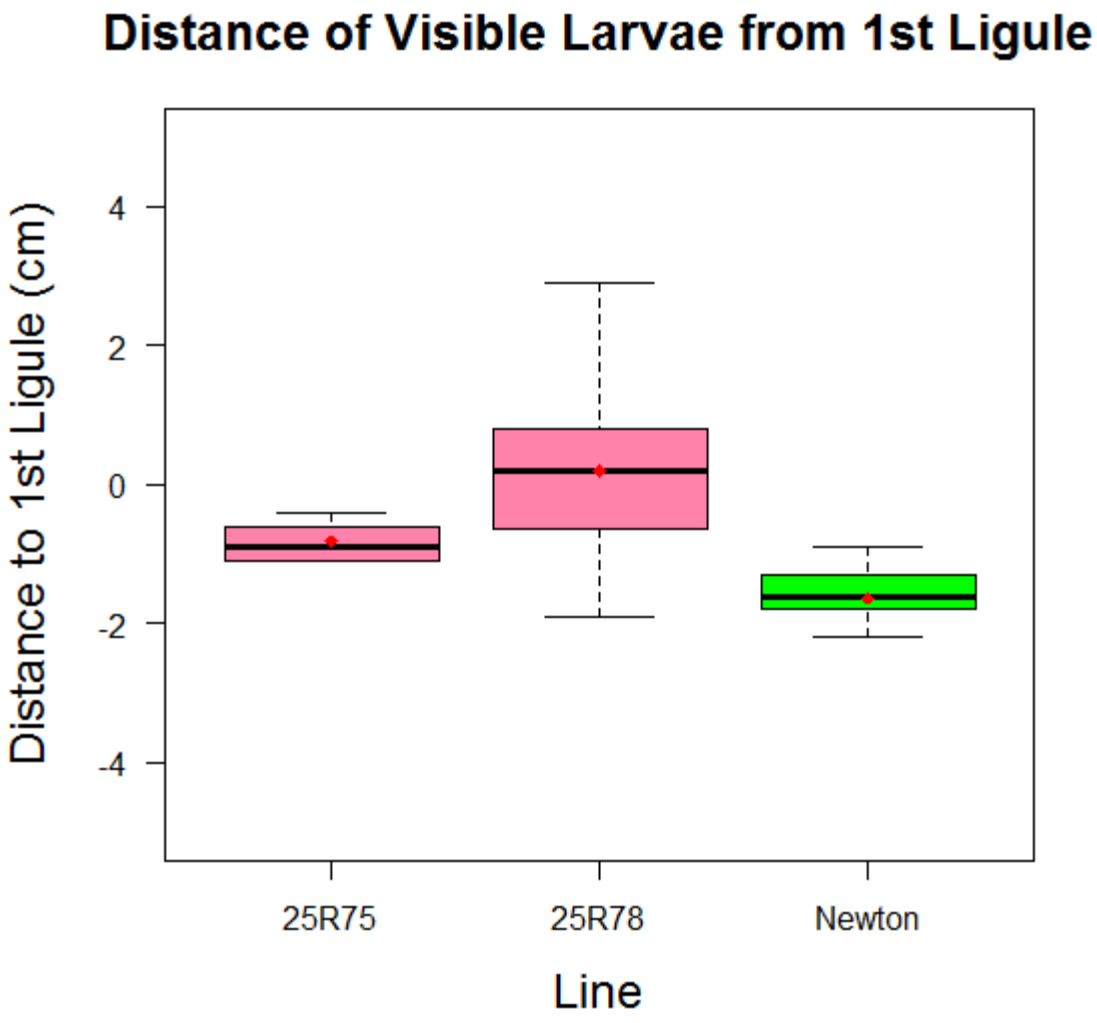

Figure 3.3 Relationship between the average distance of visible larvae from the first ligule per plant, wheat line, and treatment. The box plot highlights the sample mean (red dot), median (thick dark line), first and third quartiles (lower and upper edges), and minimum and maximum (lower and upper range bars). Possible outliers in a sample are indicated by the open circles.

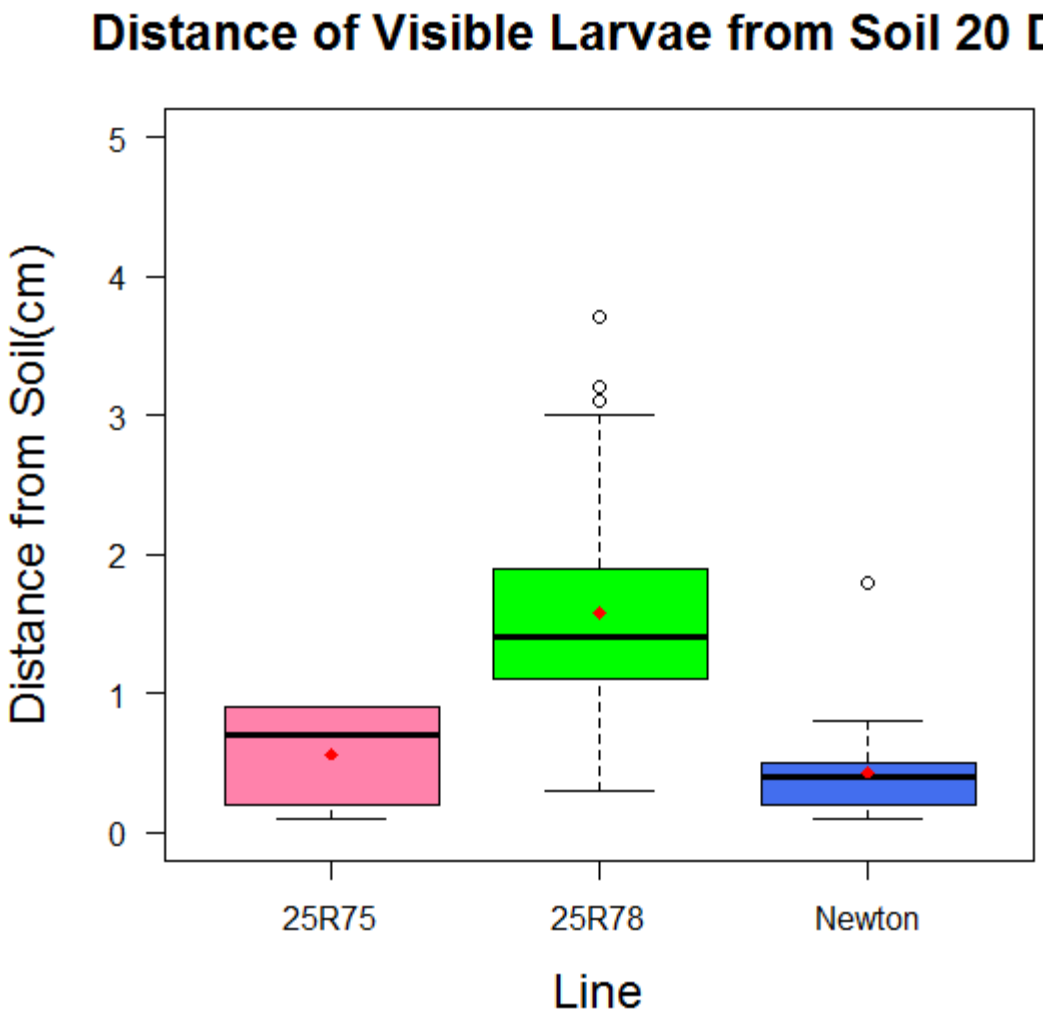

Figure 3.4 Relationship between the average distance of visible larvae from soil per plant, wheat line, and treatment. Treatments where the average distance of visible larvae from the soil per plant differ significantly ($P < 0.05$) from their corresponding controls are indicated by a black asterisk. The box plot highlights the sample mean (red dot), median (thick dark line), first and third quartiles (lower and upper edges), and minimum and maximum (lower and upper range bars). Possible outliers in a sample are indicated by the open circles.

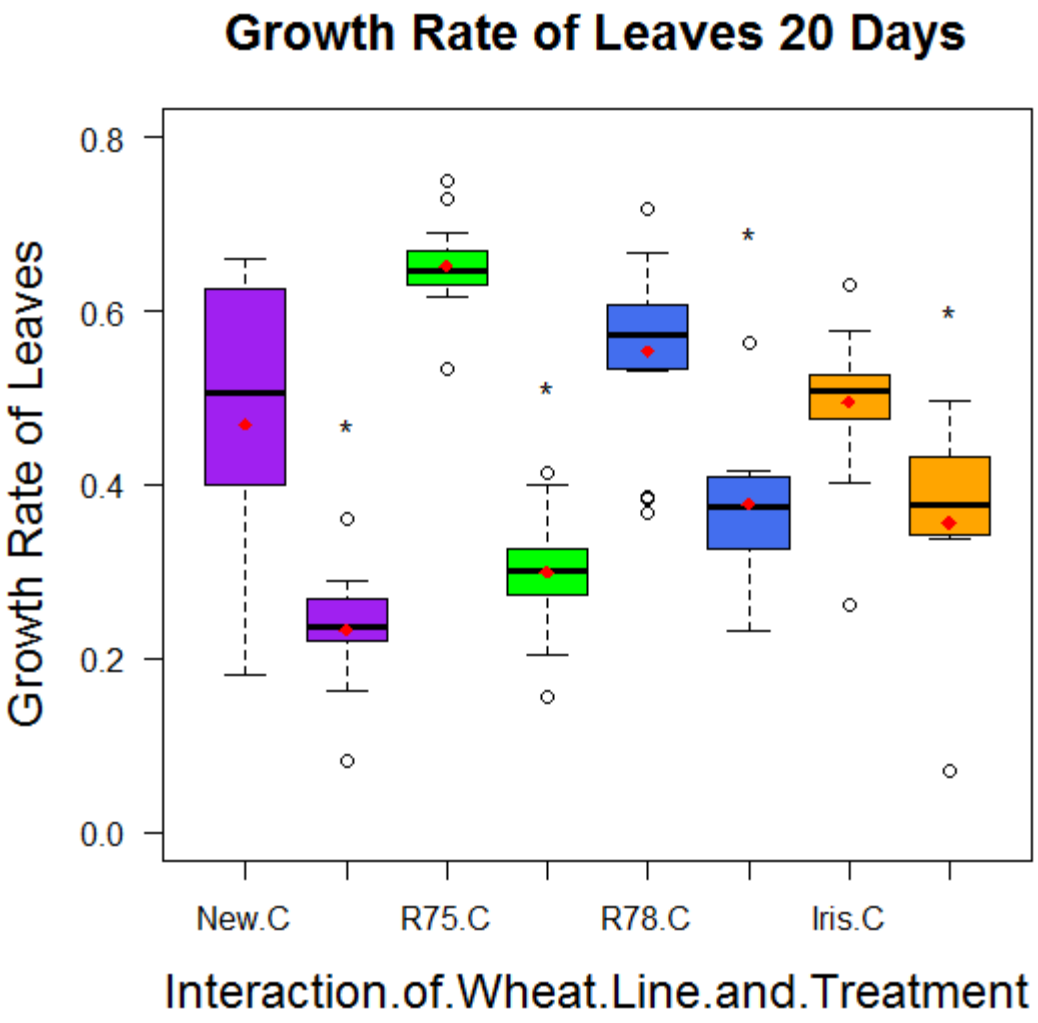

Figure 3.5 Relationship between the growth rate of leaves per plant, wheat line and treatment. Treatments where growth rate of leaves per plant means differ significantly ($P < 0.05$) from their corresponding controls are indicated by a black asterisk. The box plot highlights the sample mean (red dot), median (thick dark line), first and third quartiles (lower and upper edges), and minimum and maximum (lower and upper range bars). Possible outliers in a sample are indicated by the open circles.

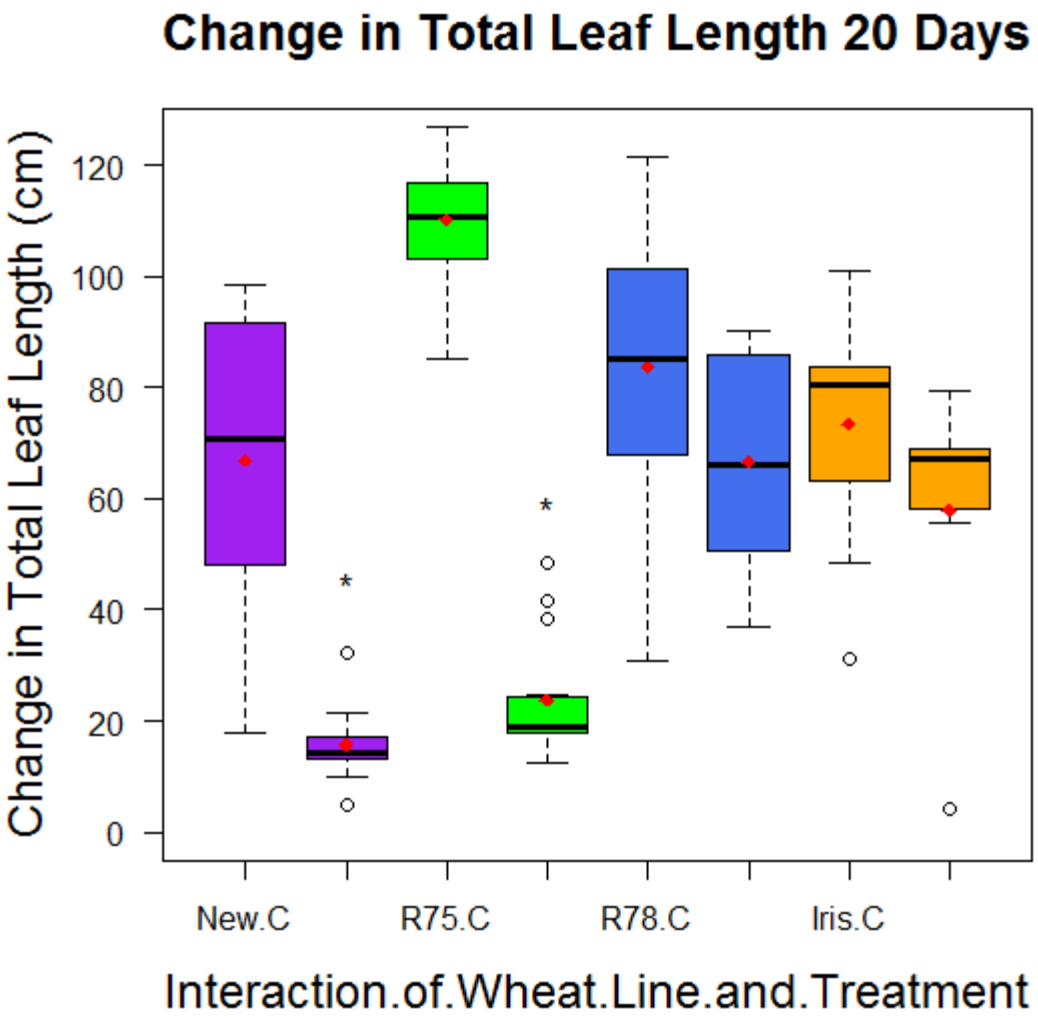

Figure 3.6 Relationship between the change in total leaf length per plant, wheat line and treatment. Treatments where change in total leaf length per plant means differ significantly ($P < 0.05$) from their corresponding controls are indicated by a black asterisk. The box plot highlights the sample mean (red dot), median (thick dark line), first and third quartiles (lower and upper edges), and minimum and maximum (lower and upper range bars). Possible outliers in a sample are indicated by the open circles.

CHAPTER 4. EFFECTS OF HESSIAN FLY INFESTATION ON TOLERANT WHEAT YIELD

4.1 Abstract

In chapters 2 and 3, the effects of Hessian fly infestation on plant growth as well as the effects of tolerant plants on the larvae were studied. Plant growth effects at the younger plant stages were previously studied by looking at leaves during three time intervals: 16, 20, and 32 dpi. The results indicated that the tolerant plants were capable of reducing or preventing different aspects of damage (i.e. leaf and tiller number, leaf length). In order to understand if these reduced growth effects translate into reduced yield effects and to analyze the economic feasibility of tolerant wheat as a control method, this study focused on analyzing the effect of infestation on tolerant wheat yield. This study analyzed tolerant Pioneer variety 25R78, resistant Pioneer brand variety 25R32, and susceptible Pioneer brand variety 25R47 through harvest. Twenty-six plants were planted for each treatment for a total of 156 plants. Plants were infested using a plastic cover and allowing 1-2 female flies to lay eggs for two hours. Measurements included height of the tallest leaf, height of the tallest head, total head height, total stem length, head lengths, head and fertile head number, and tiller number. Seeds were analyzed by measuring total seed number and weight as well as average seed number and weight. The results indicate

that there was no significant effect on yield or yield components for any of the measurements for the tolerant plants. The infested tolerant plants appeared to be comparable in yield to infested resistant plants. The lack of yield loss makes tolerant wheat a usable tool in managing Hessian flies.

4.2 Introduction

The Hessian fly, or *Mayetiola destructor*, is one of the most detrimental pests of wheat (*Triticum aestivum*) worldwide. Bread wheat is a valuable crop, making up the greatest source of food calories and being the most widely cultivated crop (Fisher, 2009). However, Hessian fly infestations can lead to losses of up to $100 million per year in the United States (Cartwright and Jones, 1953). The Hessian fly range includes the wheat-growing regions of North America, Europe, and North Africa (El Bouhssini, 1996; Harris et al., 2003; Barnes, 1956). In Morocco, heavy infestations can cause complete crop loss and, at other times, can cause 32–36% of yield loss (El Bouhssini, 1996; Amri et al., 1992; Lhaloui et al., 1992b).

The Hessian fly is in the gall midge family (Cecidomyiidae), but induce nutritive tissue instead of a normal gall (Harris et al., 2003). The first and second instar feed at this site for a total of 10–14 days (Gallun and Langston, 1963; Gagne and Hatchett, 1989). At the feeding site in compatible interactions, cells accumulate organelles and free amino acids, cell walls thin, and cells rupture (Harris et al., 2006). The breakdown of epidermal and mesophyll cells releases nutrients for the larvae to ingest. Larval feeding can stunt plants irreversibly, reduce stem elongation, prevent nutrient allocation to the developing head of grain, and even kill the plant (Buntin, 1999). However, in incompatible

interactions, feeding sites fail to form and larvae die after 3–5 days (Ratcliffe and Hatchett, 1997; Agrios, 1997; Harris et al., 2006; Shukle et al., 1990). These incompatible interactions are due to resistance genes in the plant. There are currently 35 resistance genes identified (Sardesai et al., 2005; Li et al., 2013; McDonald et al., 2014). Resistance genes are currently the primary method of Hessian fly control. However, the lethal larval antibiosis places selection pressures on fly populations, leading to an increased frequency of virulent biotypes that are capable of overcoming resistance genes.

One possible solution would be to combine the ability to survive initial infestation and the absence of selection pressures on fly populations. This might be possible with tolerance in wheat to Hessian fly damage. Tolerance, in general, is the ability for a plant to recover, grow, and potentially reproduce despite pest attack, without placing selection pressures on the pest populations (Reese et al., 1994; Strauss and Agrawal, 1999). These plants tolerate damage, instead of harming or killing the pests (Painter, 1951).

In the previous studies, the effect of infestation on tolerant plant growth (at 16, 20, and 32 dpi) and the effect of tolerant plant growth on larval position was studied. These experiments were aimed to understand if Pioneer variety 25R78 was indeed tolerant and what that tolerance would do in regards to protecting the plant and affecting Hessian fly larvae. The effects on plant growth in regard to leaf length and number are important to study permanent, progressive growth effects such as deficits or surpluses. However, this study was key to understanding whether infestation of tolerant varieties would cause any permanent growth effects on yield. This knowledge would be important for farmers who would use the Pioneer variety 25R78.

4.3 Materials and Methods

4.3.1 Plant and Insect Preparation

The lines used were susceptible Pioneer brand variety 25R47, tolerant Pioneer brand variety 25R78, and resistant Pioneer brand variety 25R32. Each line had an uninfested control and an infested treatment. The planting was staggered across subsequent days with 25R32, 25R47, and 25R78 planted in that order. In a greenhouse, square pots (3.10 cm. x 3.10 cm. x 2.33 cm., Traditional Inserts, and 18 pots/packs per insert) were filled with soil to within 2.54 cm. of the rim. Water was added and the soil was mixed until no dry soil remained. After the pots drained, one seed was planted for each pot and gently pressed into the soil. Twenty-six seeds were planted for each line and treatment (6 sets total). More soil was mixed with water and 1 cm of this soil was added to each pot over the seed. Each pot was watered twice with only water to the rim. Then, they were watered with a solution of 2,300 mL and 5.48 g of fertilizer (Scotts General Purpose, 20:20:20).

Hessian fly biotype E (avirulent on H3 Monon) was maintained by Sue Cambron at the USDA-ARS Crop Production and Pest Control Research Unit, Purdue University according to the procedure described by Foster et al. (1988). The flies were maintained in a 4°C cold storage unit. Wheat material with puparia was removed from the unit and placed in a clear plastic box (26 by 39 cm), moistened with water, and kept under fluorescent lights at 18°C for 11 days.

4.3.2 Plant Infestation

After 11 days when the plants were at the 2- to 3- leaf stage, plants were brought into the lab and measurements were taken. Then, the plants were covered with plastic covers (24 oz. Meijer brand water bottles) (Fig. D.1). These covers had 2.54 cm. of the bottom removed, a hole (2.54 cm. diameter) cut on the side, and mesh hot-glued to the top (with the lid removed). One mated female fly was placed with an aspirator in each cover and a styrofoam plug was placed in the hole. Flies remained for 6 hours and were then removed. Egg numbers were counted and any plants in the treatment group that had no eggs or larvae present were removed from the analysis.

The pots were kept inside under a fluorescent light at 18°C for 5 days. On the 5th day, the covers were removed and the pots were placed in the growth chamber at 16°C (± 2°C). Each pot was watered with 200 mL tap water 4–5 days per week. The pots were each watered for 4 seconds after 16 days in the growth chamber with a solution of 2,300 mL and 5.48 g. of fertilizer (Scotts General Purpose, 20:20:20). The plants remained in the growth chamber for 35 days. The pots were randomly placed in groups of six with in the chamber. Each group of six had one pot for each factor level (i.e. one pot from each line and for both treatments). At the 16-day mark, they were watered with fertilizer water as above. After 35 days, the plants were placed in a cold storage unit (Bally) for 64 days (4°C). Watering occurred 1–2 times a week as needed. Every plant was sprayed as needed with fungicide to treat powdery mildew.

4.3.3 Plant Transplant

After 65 days, the plants were placed in a growth chamber (Revco Honeywell, 16°C, 80% humidity) for 15 days. Then, the plants were placed in a greenhouse for 29 days. Chambers were placed over the plants after 24 days to catch emerging adult flies (Fig. D.1). Then, after 5 days, the chambers were removed and each plant was transferred to an individual pot (15.24 cm. diameter, 15.24 cm. deep) with moist soil. The moist soil was previously mixed in each pot so that it reached to within 10 cm of the rim. A large scoop of soil was removed in the center, forming a valley 10 cm across and 25 cm in depth, and 7–10 fertilizer pellets were placed in a 3 cm diameter circle in the hollow.

Then, 1.5 cm. of moist soil from around the sides was placed on top and was pressed down, forming a flat surface. The plant, roots, and soil was removed from each square pot and placed gently in the hole left in the new pot. Extra moist soil was placed around the sides of the plant to fill in any spaces. The plant was watered with tap water twice. For the next 12 weeks, plants were watered 5 days a week with 200 mL of water. After the first head was 50% emerged for each plant, the number of heads was recorded. Nine days later, the number of heads and height of the tallest leaf was measured for that plant. For the last 3 weeks, plants were watered 3 days a week and treated with insecticide (Ortho Flower, Fruit & Vegetable) for aphid control. Each line was harvested 15 weeks after transplanting.

4.3.4 Measurements Taken

The measurements used were modeled after the research on the fitness costs of *H*-gene mediated resistance in wheat to Hessian flies (Anderson et al., 2011). The measurements recorded included the following for each plant: plant survival, head number, fertile head number, seed number, seed weight, head height, head length, tallest leaf length, tiller number, and stem length (for those with heads). Using a clear ruler pressed into the soil, the tallest leaf height was measured from the leaf tip to the first ligule. Measurements were rounded down to the nearest millimeter. Head height was measured from the soil to the head tip (excluding awns). Head length was measured from the bottom of the head to the tip (excluding awns). The stem length was measured from the soil to the bottom of the head. Fertile head number included only the heads with seeds. Seed weight was measured using a scale (College B303-S, Mettler Toledo). The seeds for each individual head were placed together on a piece of circular filter paper (Whatman, 12.5 cm, Grade 5). Average seed number was calculated by taking the average of the seed numbers from each head of a plant. The same was done for average seed weight. Total seed number and weight was taken by finding the sum of either the count or weight of all seeds from a plant.

The growth rate of the leaves at the stem was measured in several steps. First, each leaf was measured on the day of infestation and 20 dpi. The leaf was measured from the tip to the collar of the first ligule to control for movement of soil over time due to watering. The change in height was calculated by subtracting the initial measurement for each leaf from the final measurement. Then, this value was divided by 20 to calculate

relative growth rate based on the number of days. The growth rates of all the leaves of a given plant were summed together and divided by the total number of leaves. This resulted in the overall average growth rate of leaves per plant. Dividing by the number of days allows for the measurement to be compared to other studies with similar setups, but with different time intervals.

4.3.5 Statistical Analysis

Statistical analyses were performed in R (R Core Team, 2014). The design was a two-way factorial with the following levels: (1) infested 25R32, (2) uninfested 25R32 as control, (3) infested 25R47, (4) control uninfested 25R47, (5) infested Pioneer variety 25R78, and (6) uninfested control Pioneer variety 25R78. The sample sizes for the different levels were the following: control Pioneer variety 25R78 (n = 24), treated Pioneer variety 25R78 (n = 27), control 25R47 (n = 27), treated 25R47 (n = 16), control 25R32 (n = 28), and treated 25R32 (n = 26). A statistical significance threshold of α = 0.05 was used.

Change in total leaf length, average leaf length, and larvae number were continuous variables with a normal distribution while leaf and tiller numbers were integer ratio scale variables. Each of these variables was analyzed using a two-way ANOVA. The model was made using the *aov* function of package in R (R Core Team, 2014). Plant survival was a categorical variable, analyzed using a two-way Chi-square test in R.

4.4 Results and Discussion

4.4.1 Number of Heads and Fertile Heads

The treatment (F-value = 9.650, P = 0.002), line (F-value = 3.657, P = 0.028), and interaction between the two (F-value = 3.570, P = 0.031) appeared to have a significant impact on the number of heads (Table 4.1). The difference between infested and uninfested resistant Pioneer 25R32 was not significant (P = 0.790) (Fig. 4.1). The difference between infested and uninfested tolerant Pioneer variety 25R78 was also not statistically significant (P = 0.999). However, infested susceptible Pioneer variety 25R47 was significantly smaller than the uninfested plants (P = 0.006). There was no significant difference in head number between infested tolerant plants and infested Pioneer variety 25R47 plants as well as infested Pioneer variety 25R32 plants (P = 0.999 and P = 0.994, respectively) (Table 4.4).

The treatment (F-value = 9.548, P = 0.002), line (F-value = 3.877, P = 0.023), and interaction between the two (F-value = 3.323, P = 0.039) appeared to have a significant impact on the number of fertile heads (Table 4.1). The difference between infested and uninfested resistant Pioneer 25R32 was not significant (P = 0.864) (Fig. 4.2). The difference between infested and uninfested tolerant Pioneer variety 25R78 was also not statistically significant (P = 0.999). However, infested susceptible Pioneer variety 25R47 was significantly smaller than the uninfested plants (P = 0.006). There was no significant difference in head number between infested tolerant plants and infested Pioneer variety 25R47 plants as well as infested Pioneer variety 25R32 plants (P = 0.999 and P = 0.969, respectively) (Table 4.4).

4.4.2 Tiller Number

The treatment (F-value = 11.48, P = 0.001) and line (F-value = 5.372, P = 0.006), but not the interaction between the two (F-value = 2.158, P = 0.119) appeared to have a significant impact on the number of tillers (Table 4.3). The difference between infested and uninfested resistant Pioneer 25R32 was not significant (P = 0.935) (Fig. 4.3). The difference between infested and uninfested tolerant Pioneer variety 25R78 was also not statistically significant (0.898). However, infested susceptible Pioneer variety 25R47 was significantly smaller than the uninfested plants (P = 0.015). There was no significant difference in head number between infested tolerant plants and infested Pioneer variety 25R47 plants or infested Pioneer variety 25R32 plants (P = 0.974 and P = 0.912, respectively) (Table 4.4).

4.4.3 Total Seed Number and Weight

The treatment (F-value = 9.400, P = 0.003), but neither the line (F-value = 2.426, P = 0.092) nor the interaction between the two (F-value = 2.703, P = 0.070) appeared to have a significant impact on the number of total seed number/plant (Table 4.2). The difference between infested and uninfested resistant Pioneer 25R32 was not significant (P = 0.678) (Fig. 4.5). The difference between total seed number for infested and uninfested tolerant Pioneer variety 25R78 was also not statistically significant (P = 0.993). However, infested susceptible Pioneer variety 25R47 had significantly fewer seeds than the uninfested plants (P = 0.007). There was no significant difference in total seed number between infested tolerant plants and infested Pioneer variety 25R32 plants or infested Pioneer variety 25R47 plants (P = 0.933 and P = 0.492, respectively) (Table 4.5).

The treatment (F-value = 13.42, P = < 0.001), but not the line (F-value = 0.069, P = 0.933), and the interaction between the two (F-value = 2.638, P = 0.075) appeared to have a significant impact on the number of total seed weight/plant (Table 4.2). The difference between infested and uninfested resistant Pioneer 25R32 was not significant (P = 0.450) (Fig. 4.6). The difference between infested and uninfested tolerant Pioneer variety 25R78 was also not statistically significant (P = 0.960). However, infested susceptible Pioneer variety 25R47 was significantly smaller than the uninfested plants (P = 0.003). There was no significant difference in total seed number between infested tolerant plants and infested Pioneer variety 25R32 plants or infested Pioneer variety 25R47 plants (P = 0.999 and P = 0.681, respectively) (Table 4.5).

4.4.4 Average Seed Number and Weight

The line (F-value = 5.776, df = 2, P = 0.004), but not treatment (F-value = 0.181, df = 1, P = 0.672) or interaction of the two (F-value = 0.009, df = 2, P = 0.991) appeared to have a significant impact on the average seed number/plant (Table 4.2). There appeared to be no significant difference in average seed number between infested and uninfested tolerant plants (P = 0.991) (Fig. 4.4). The same is true for Pioneer 25R32 and Pioneer variety 25R47 (P = 0.997 and P = 0.999, respectively). Infested tolerant plants showed significantly more seeds than infested Pioneer 25R47 plants (P = 0.018). There was no significant difference between average seed numbers of infested tolerant and resistant plants (P = 0.999).

The line (F-value = 13.82, df = 2, P = < 0.001), but not treatment (F-value = 0.040, df = 1, P = 0.842) or interaction of the two (F-value = 0.095, df = 2, P = 0.910)

appeared to have a significant impact on the average seed weight/plant (Table 4.2). There appeared to be no significant difference in average seed weight between infested and uninfested tolerant plants (P = 0.992) (Fig. 4.7). The same is true for Pioneer 25R32 and Pioneer variety 25R47 (P = 0.999 and P = 0.980, respectively). There was no significant difference between average seed weights of infested tolerant and resistant plants (P = 0.729) or susceptible plants (P = 0.275).

4.5 Discussion

Infestation in tolerant plants appeared to have no permanent effects on yield, similar to infested resistant plants. The infested and uninfested tolerant plants had no significant differences in head number, fertile head number, or tiller number. Also, there was no significant difference in total seed number, total seed weight, average seed number, average seed weight, or tallest head height. The same was true between infested and uninfested resistant plants. The susceptible line, on the other hand, showed significant yield effects. Infested susceptible plants had significantly fewer heads, fertile heads, total seed number, total seed weight, and tillers.

However, there were no significant differences in tallest head height, average seed weight, or average seed number. Even with these results, it was clear that infestation affected the yield of the susceptible plants, but not the tolerant or resistant plants. The measurements that showed significant differences for the susceptible plants were those that were used by Anderson et al. (2011). That study analyzed fitness costs for *H*-gene mediated resistance by looking at yield effects of infestation (Anderson et al., 2011).

Although this experiment was focused on yield effects in tolerant plants and not fitness costs, the measurements are a useful tool for looking at yield loss.

The yield components, head and fertile head number, was not affected by infestation on tolerant and resistant plants. Even though infested susceptible plants showed significantly fewer heads than their controls, uninfested susceptible plants had significantly more heads than uninfested tolerant plants ($P = 0.004$), but not significantlyy more heads than uninfested resistant plants ($P = 0.286$). The results indicate that the tolerant line, whether infested or not, has significantly fewer heads than uninfested susceptible plants and slightly fewer heads than uninfested 25R32. This might be due to phenotypic difference in the wheat line pedigrees. Overall, the absence of a long-term growth effect on head number for resistant or tolerant plants indicates the ability for tolerant plants to tolerate larval damage.

There appeared to be no larval effects on tiller number for tolerant and resistant plants and negative effects on tiller number for susceptible plants (Table 4.3). The number of tillers per plant is one of the primary contributors to wheat grain yield (Gupta et al., 1999; Chowdhry et al. 2000). Due to this connection between tiller number and grain yield, tiller loss from Hessian fly infestation could reduce yield. In the case of tolerant Pioneer variety 25R78, the prevention of tiller loss might prevent yield loss, making the tolerant line more economically appealing to farmers. Results also indicate that infestation does not affect seed production through seed number or seed weight per head. Each of the infested lines showed no significant differences in total seed number or total seed weight compared to the corresponding uninfested plants. This indicates that

this measurement of yield showed no adverse effects from infestation, regardless of wheat line. It would be interesting to see the effect on germination or protein content.

The measurements that showed significant differences for the susceptible plants were those used by Anderson et al. (2011). Anderson et al. analyzed fitness costs for *H*-gene mediated resistance by looking at yield effects of infestation. Although this third study was focused on yield effects in tolerant plants and not fitness costs, the measurements are a useful tool at looking at yield loss since seed number per plant is another key measurement for grain yield (Gupta et al., 1999; Chowdhry et al., 2000).

There was no significant difference between the tallest head heights or average head heights of infested tolerant and susceptible plants while infested tolerant plants showed significantly smaller head heights than resistant plants (Appendix C; Table 4.4). This might indicate that tolerant plants, whether infested or uninfested, show shorter heads than the resistant plants. The height characteristics might be due to the breeding process, leading to shorter heads. Even uninfested tolerant plants showed significantly smaller tallest head heights compared to uninfested resistant plants ($P = < 0.001$). Uninfested tolerant plants also showed significantly smaller average head heights than uninfested resistant plants ($P = < 0.001$). However, since there was no significant difference in head height between infested and uninfested tolerant plants, there appeared to be no infestation effect on head height (Appendix C). The lack of significant differences within each wheat line set indicates that infestation does not affect the height of the tallest head.

No negative effects of infestation were observed for the average seed number or weight per head for any line. These results indicate that larval attack does not affect these

measurements, only the number of heads and total seed number. This varies from the results of Anderson and Harris (2011), in which the susceptible line ‘Newton’ demonstrated a negative larval effect on the average seeds per plant. The absence of larval effects on seed number is important for yield. More seeds could result in a greater yield per plant and per hectare. No larval growth effects on the tallest leaf were found for any of the lines (Appendix C). However, there were genotype effects between resistant and tolerant plants (Table 4.4). This genotype difference has been studied in other wheat lines and even among resistant lines, there can be variation in growth and yield benefits (Anderson et al., 2011).

Infested susceptible Pioneer variety 25R47 was the only line and treatment to have any dead plants with ten plants. However, plant survival was not dependent on wheat line and treatment ($P = 0.221$) (Appendix C). The presence of dead plants for the susceptible line, although not significantly, might indicate the possibility that larvae could kill susceptible plants, but not resistant or tolerant plants.

4.6 Conclusion

The yield measurements, including the primary contributors, tiller number, seed number, and seed weight, showed no effects from infestation in the tolerant plants. These wheat plants showed no significant growth effect from infestation on leaf number, head number, fertile head number, average head length, total seed number and weight, average seed number and weight, head height, and tallest leaf height. There was no yield loss for infested tolerant plants and these plants showed similar results in yield measurements compared to infested resistant plants. The only measurements showing a significant

difference between infested tolerant and infested resistant plants were the tallest leaf length, tallest head height, and average head height. However, the same was true for uninfested tolerant versus uninfested resistant plants. This might be caused by the wheat line itself, not the effect of infestation.

Overall, the tolerant Pioneer wheat variety 25R78 appears to have the ability overcome infestation with no negative effects on yield and with no plant death, similar to that of resistant plants. This, along with the possibility of the absence of selection pressure on fly populations, could make tolerance a useful method of Hessian fly control. Further research is needed to confirm these observations on a larger scale.

4.7 References

Agrios, G.N. 1997. Plant diseases caused by Mollicutes: Phytoplasmas and spiroplasmas, pp 457–470. *In*: Agrios, G.N. (Ed.), Plant Pathology, Academic Press, New York.

Amri, A, M. El Bouhssini, S. Lhaloui, T.S. Cox, J.H. Hatchett. 1992. Estimates of yield loss due to Hessian fly (Diptera: Cecidomyiidae) on bread wheat using near-isogenic lines. Al Awamia 77: 75–87.

Anderson, K.M, Q. Kang, J. Reber, M.O. Harris. 2011. No fitness cost for wheat's *H* gene-mediated resistance to Hessian Fly (Diptera: Cecidomyiidae). J. Econ. Entom. 104(4): 1393–1405.

Barnes, H.F. 1956. Gall midges of economic importance. Vol. VII: Gall midges of cereal crops. Crosby Lockwood & Son, London, UK.

Buntin, G., J.W. Chapin. 1990. Biology of Hessian fly (Diptera: Cecidomyiidae) in the southeastern United States: geographic variation and temperature-dependent phenology. J. Econom. Entomol. 83: 1015–1024

Buntin, G. D. 1999. Hessian Fly (Diptera: Cecidomyiidae) injury and loss of winter wheat grain yield and quality. J. Econ. Entomol. 92: 1190–1197.

Cartwright, W.B., E.T. Jones. 1953. The Hessian fly and how losses from it can be avoided. USDA Farmers' Bul. 162. 1–9.

Chowdhry, M.A., M. Ali, G.M. Subhani, I. Khaliq. 2000. Path coefficient analysis for water use efficiency, evapo-transpiration efficiency, transpiration efficiency and some yield related traits in wheat. Pak. J. Biol. Sci. 3: 313–317.

El Bouhssini, M., S. Lhaloui, A. Amri, M. Jlibene, J.H. Hatchett, N. Nssarellah, M. Nachitt. 1996. Wheat genetic control of Hessian fly (Diptera: Cecidomyiidae) in Morocco. Field Crops Res. 45: 111–114.

Fisher, R.A. 2009. Foreword, p. xvii. *In*: B.F. Carver (Ed.), Wheat science and trade, Wiley-Blackwell, Ames, IA.

Gallun, R.L., R. Langston. 1963. Feeding habits of Hessian fly larvae on ^{32}P-labelled resistant and susceptible wheat seedlings. J. Econ. Entomol. 56: 702–706.

Gagne, R., J.H. Hatchett. 1989. Instars of the Hessian fly. Ann. Entomol. Soc. Am. 82: 73–79.

Gupta, A.K., R.K. Mittal, A. Ziauddin. 1999. Association and factor analysis in spring wheat. Ann. Agric. Res. 20: 481–485.

Harris, M.O., J.J. Stuart, M. Mohan, S. Nair, R.J. Lamb, O. Rohfritsch. 2003. Grasses and gall midges: Plant defense and insect adaptation. Annu. Rev. Entomol. 48:549–577.

Harris, M.O., T.P. Freeman, O. Rohfritsch, K.G. Anderson, S.A. Payne, J.A. Moore. 2006. Virulent Hessian Fly (Diptera: Cecidomyiidae) larvae induce a nutritive tissue during compatible interactions with wheat. 99(2): 305–316.

Lhaloui, S., L. Buschman, M. El Bouhssini, K. Starks, D. Keith, K. El Houssaini. 1992. Control of Mayetiola species (Diptera: Cecidomyiidae) with carbofuran in bread wheat, durum wheat, and barley, with yield loss assessment and its economic analysis. Al Awamia. 77: 55–73.

Li, C., M. Chen, S. Chao, J. Yu, G. Bai. 2013. Identification of a novel gene, *H34*, in wheat using recombinant inbred lines and single nucleotide polymorphism markers. TAG. 8: 2065–2071.

McDonald, M., H. Ohm, K. Rinehart, M. Giovanini, C. Williams. 2014. *H33*: A Wheat Gene Providing Hessian Fly Resistance for the Southeastern United States. Crop Science. 54: 2045–2053.

Ratcliffe, R.H., J.H. Hatchett. 1997. Biology and genetics of the Hessian fly and resistance in wheat, pp. 47–56: *In* Bondarie, K. (Ed.), New Developments in Entomology, Research Signpost. Scientific Information Guild, Trivandrum, India.

Reese, J., J. Schwenke, P. Lamont, D. Zehr. 1994. Importance and Quantification of Plant Tolerance in Crop Pest Management Programs for Aphids: Greenbug Resistance in Sorghum. J. Agr. Entomol. 11: 255–270.

Sardesai, N., J. Nemacheck, S. Subramanyam, C.E. Williams. 2005. Identification and mapping of H32, a new wheat gene conferring resistance to Hessian fly. Theor. Appl. Genet. 111: 1167–1173.

Shukle, R. H., P.B. Grover, J.E. Foster. 1990. Feeding of Hessian fly (Diptera: Cecidomyiidae) larvae on resistant and susceptible wheat. Environ. Entomol. 19: 494–500.

Strauss, S., A. Agrawal. 1999. The ecology and evolution of plant tolerance to herbivory. TREE. 14: 179–185.

Painter, R.H. 1951. Insect Resistance in Crop Plants. Macmillan, New York, NY, USA.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. URL http://www.R-project.org/.

Shah, W.A., H.U. Khan, S. Anwar, K. Nawab. 2011. Yield and yield components affected by different seed rates and nitrogen levels. Sarhad J. Agric. 27(1): 17-25.

4.8 Tables and Figures

Table 4.1 Effects of treatment and wheat line, and their interaction on the number of wheat heads and fertile heads as determined by two-way ANOVA.

Measurement		F-value	Df	P-value	Sum Sq.	Mean Sq.
Head Number	Treatment	9.650	1	0.002**	28.80	28.82
	Line	3.657	2	0.028*	21.80	10.92
	Treatment:Line	3.570	2	0.031*	21.30	10.66
Fertile Head Number	Treatment	9.548	1	0.002**	28.10	28.12
	Line	3.877	2	0.023*	22.80	11.41
	Treatment:Line	3.323	2	0.039*	19.60	9.786

* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$.

Table 4.2 Effects of treatment and wheat line, and their interaction on the total number and weight of seeds and the average number and weight of seeds as determined by two-way ANOVA.

		F-value	Df	P-value	Sum Sq.	Mean Sq.
Total Seed Number	Treatment	9.400	1, 142	0.003**	5029	5029
	Line	2.426	2, 142	0.092	2595	1298
	Treatment:Line	2.703	2, 142	0.070	2892	1446
Average Seed Number	Treatment	0.040	1, 142	0.842	0.600	0.650
	Line	13.82	2, 142	< 0.001***	451.5	225.8
	Treatment:Line	0.095	2, 142	0.910	3.100	1.550
Total Seed Weight (g)	Treatment	13.42	1, 142	< 0.001***	2.471	2.471
	Line	0.069	2, 142	0.933	0.025	0.013
	Treatment:Line	2.638	2, 142	0.075	0.972	0.486
Average Seed Weight (g)	Treatment	0.181	1, 142	0.672	0.002	0.002
	Line	5.776	2, 142	< 0.001***	0.117	0.058
	Treatment:Line	0.009	2, 142	0.991	0.000	0.000

* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$.

Table 4.3 Effects of treatment and wheat line, and their interaction on tiller number, height of the tallest leaf and head, and the average head height as determined by two-way ANOVA.

		F-value	Df	P-value	Sum Sq.	Mean Sq.
Height of Tallest Head (cm)	Treatment	0.680	1, 142	0.411	9.800	9.800
	Line	25.02	2, 142	< 0.001***	717.9	358.9
	Treatment:Line	0.285	2, 142	0.752	8.200	4.100
Tallest Leaf (cm)	Treatment	0.113	1, 142	0.737	1.800	1.790
	Line	10.99	2, 142	< 0.001***	346.5	173.2
	Treatment:Line	0.275	2, 142	0.760	8.700	4.340
Average Head Height (cm)	Treatment	0.907	1, 142	0.342	12.20	12.20
	Line	28.54	2, 142	< 0.001***	766.7	383.4
	Treatment:Line	0.148	2, 142	0.863	4.000	2.000
Tiller Number	Treatment:Line	2.158	2, 142	0.119	14.70	7.340
	Treatment	11.48	1, 142	< 0.001***	39.00	39.04
	Line	5.372	2, 142	0.006**	36.50	18.27

* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$.

Table 4.4 Post-hoc Tukey analysis on head number, tiller number, height of the tallest leaf, height of the tallest head, and average head height.

Measurement	Wheat Line	P value
Head Number	25R78-25R47	0.994
	25R78-25R32	0.999
Tiller Number	25R78-25R47	0.974
	25R78-25R32	0.912
Height of Tallest Leaf	25R78-25R47	0.401
	25R78-25R32	0.018*
Height of Tallest Head	25R78-25R47	0.999
	25R78-25R32	< 0.001***
Average Head Height	25R78-25R47	0.990
	25R78-25R32	< 0.001***

* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$.

Table 4.5 Post-hoc Tukey analysis on total seed number, total seed weight, average seed number, and average seed weight.

Measurement	Wheat Line	P value
Total Seed Number	25R78-25R47	0.933
	25R78-25R32	0.492
Total Seed Weight	25R78-25R47	0.999
	25R78-25R32	0.681
Average Seed Number	25R78-25R47	0.018*
	25R78-25R32	0.999
Average Seed Weight	25R78-25R47	0.275
	25R78-25R32	0.729

* Significant at $P \leq 0.05$.
** Significant at $P \leq 0.01$.
*** Significant at $P \leq 0.001$.

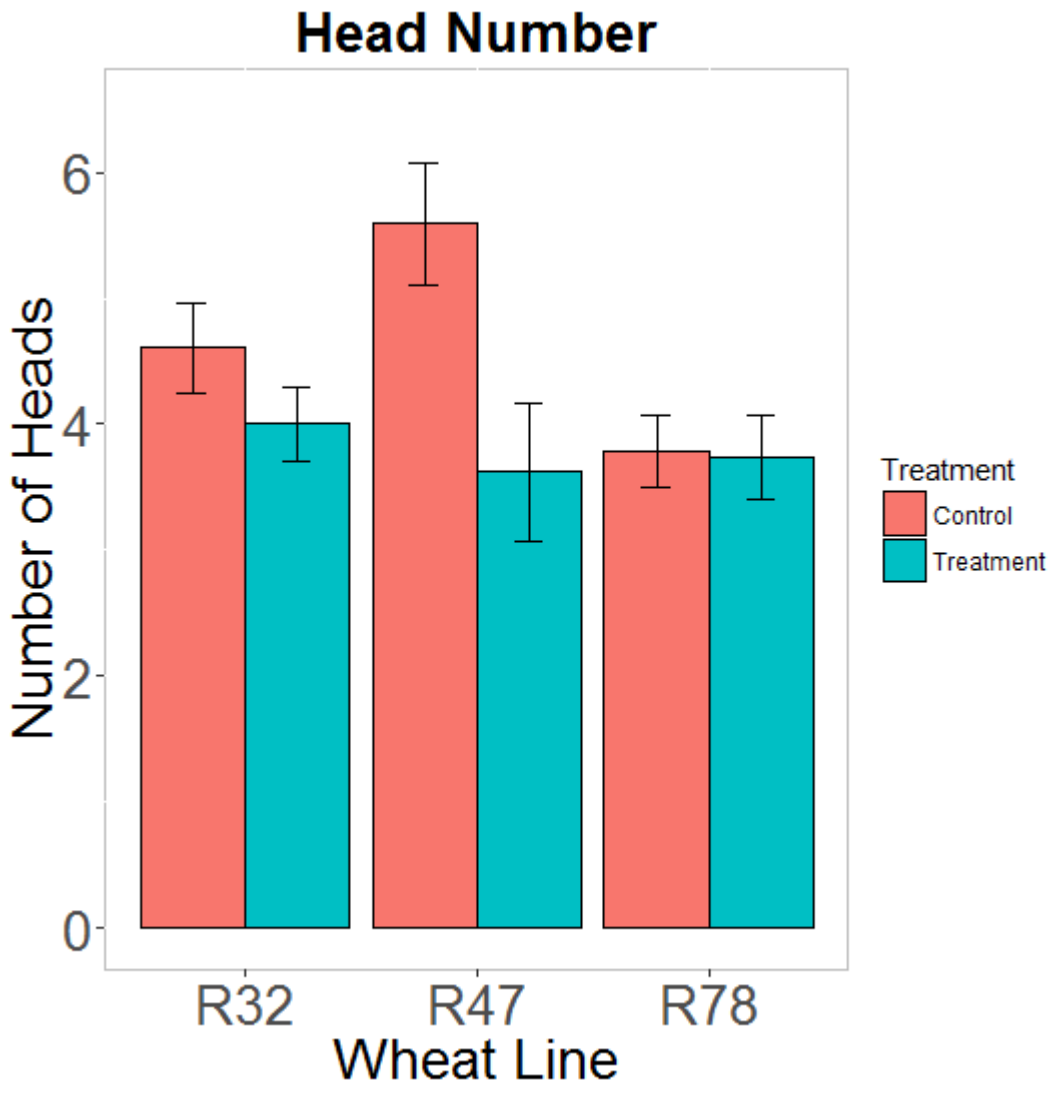

Figure 4.1 Relationship between head number, wheat line, and treatment. Wheat lines: resistant 25R32 (R32), susceptible Pioneer variety 25R47 (R47), and tolerant Pioneer variety 25R78 (R78) when infested (blue) and uninfested (pink). Error bars represent standard errors of each sample.

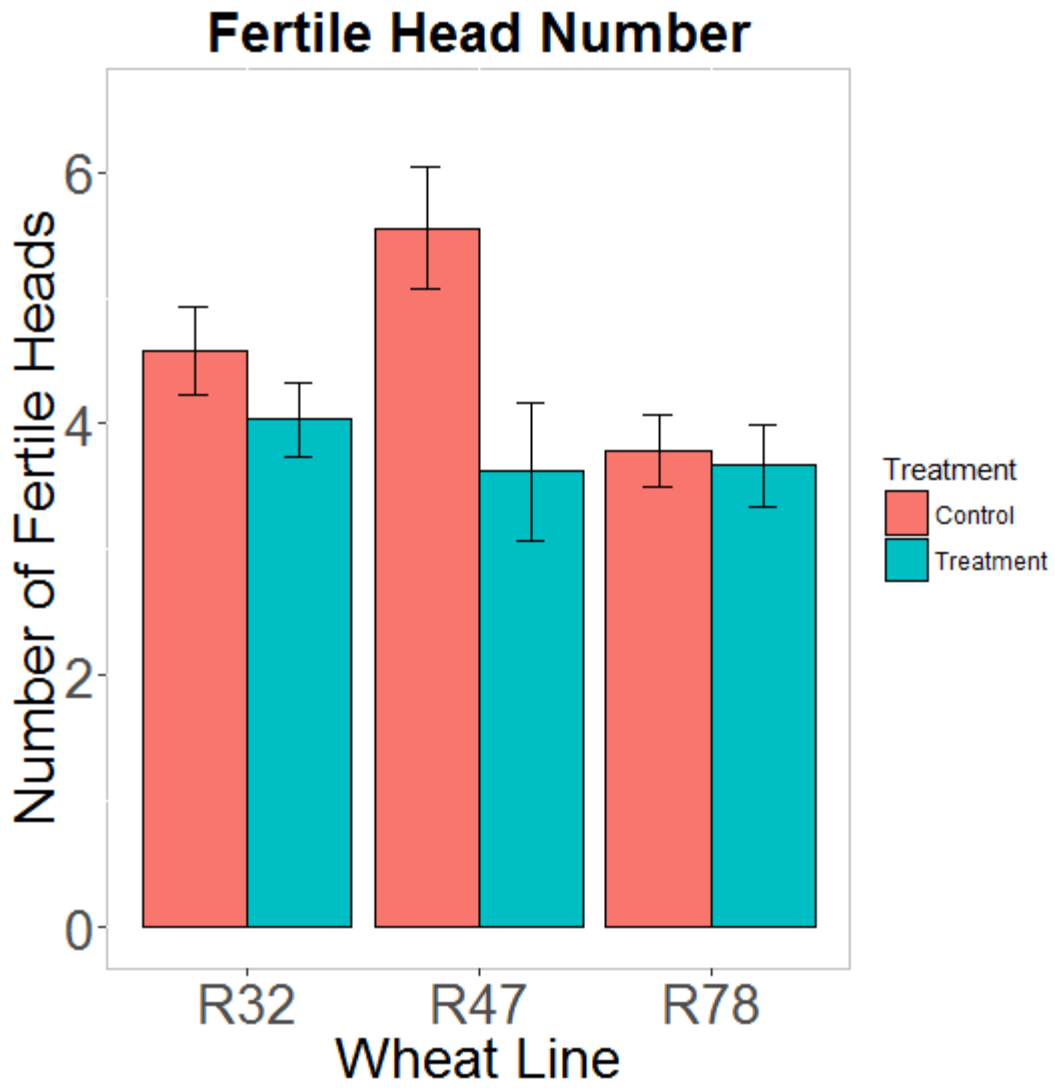

Figure 4.2 Relationship between fertile head number, wheat line, and treatment. Wheat lines: resistant 25R32 (R32), susceptible Pioneer variety 25R47 (R47), and tolerant Pioneer variety 25R78 (R78) when infested (blue) and uninfested (pink). Error bars represent standard errors of each sample.

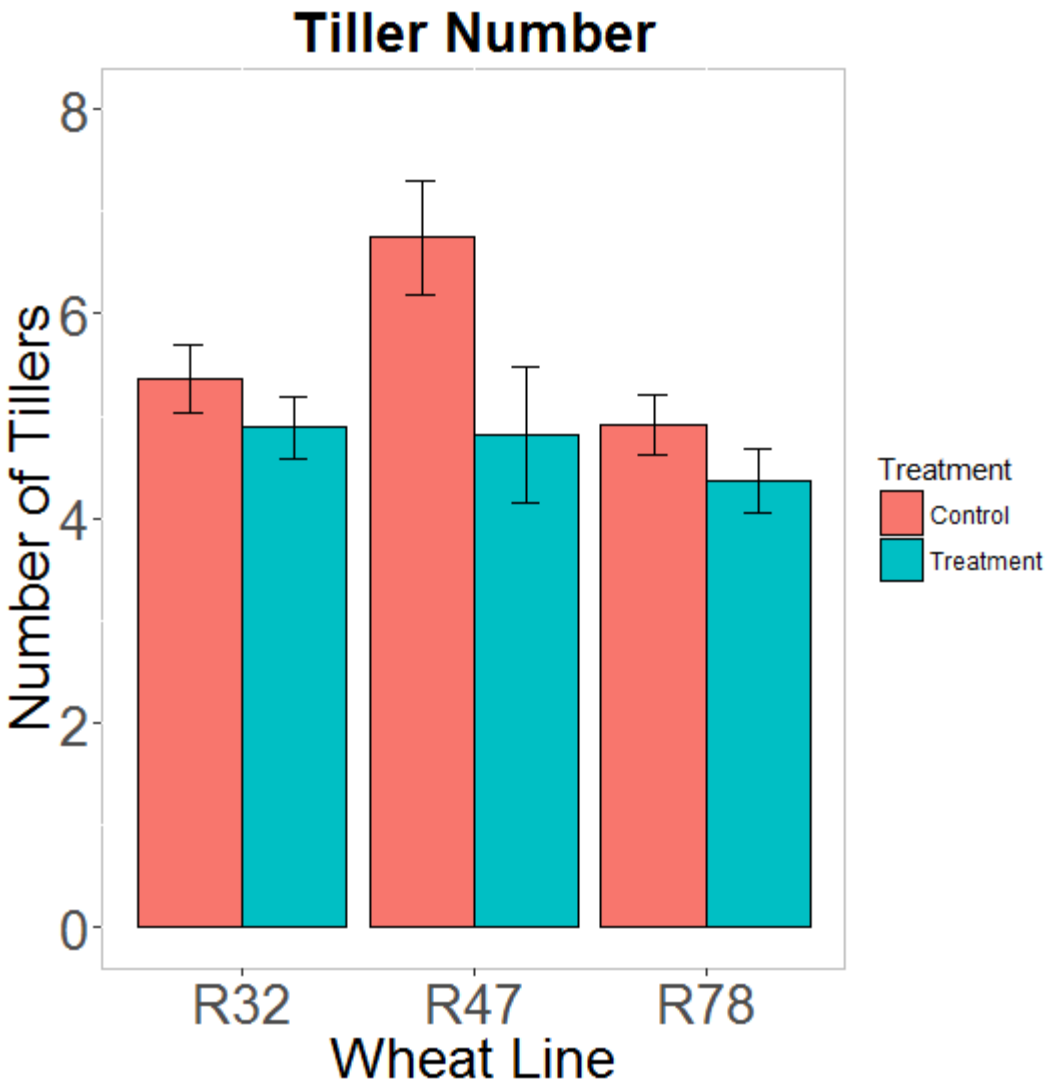

Figure 4.3 Relationship between tiller number, wheat line, and treatment. Wheat lines: resistant 25R32 (R32), susceptible Pioneer variety 25R47 (R47), and tolerant Pioneer variety 25R78 (R78) when infested (blue) and uninfested (pink). Error bars represent standard errors of each sample.

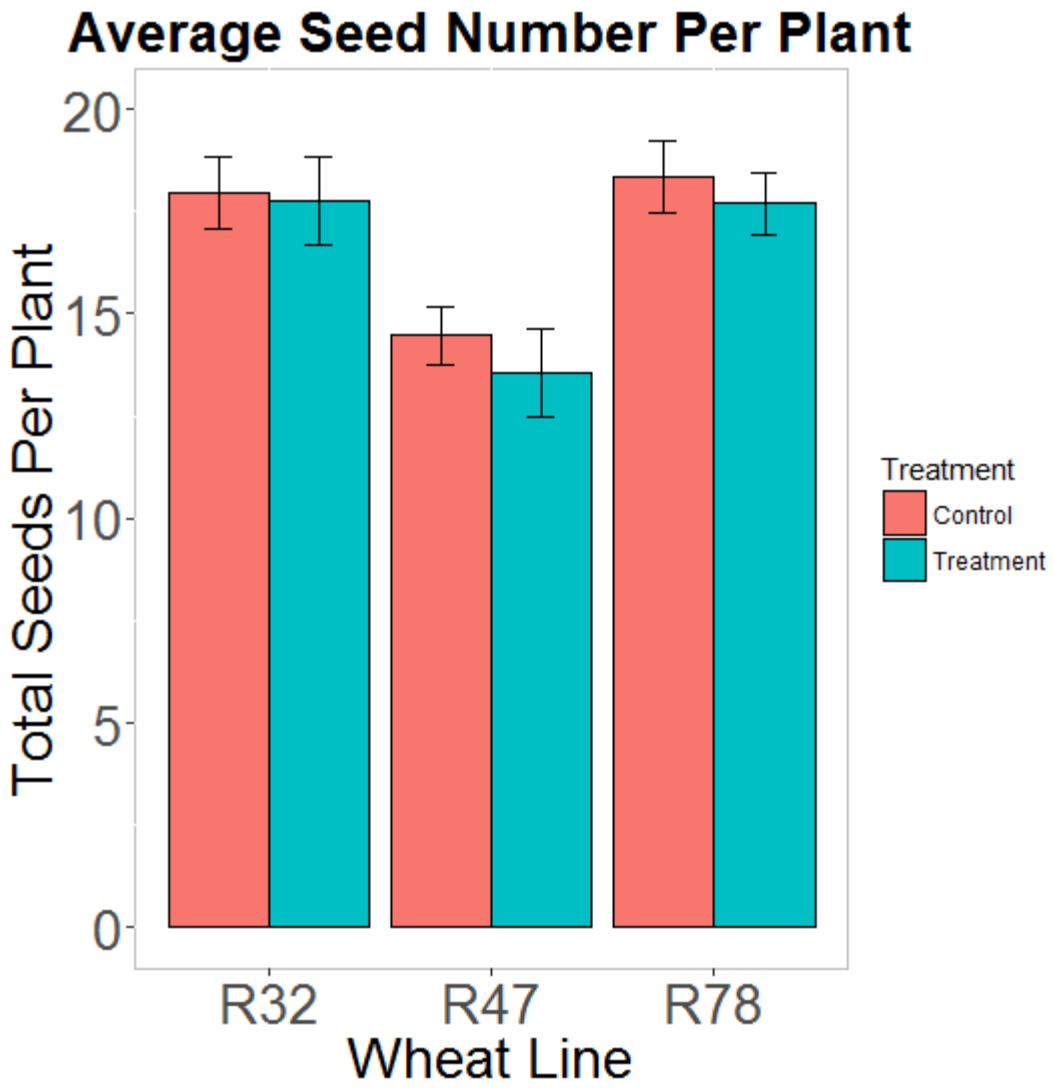

Figure 4.4 Relationship between average seed number per plant, wheat line, and treatment. Wheat lines: resistant 25R32 (R32), susceptible Pioneer variety 25R47 (R47), and tolerant Pioneer variety 25R78 (R78) when infested (blue) and uninfested (pink). Error bars represent standard errors of each sample.

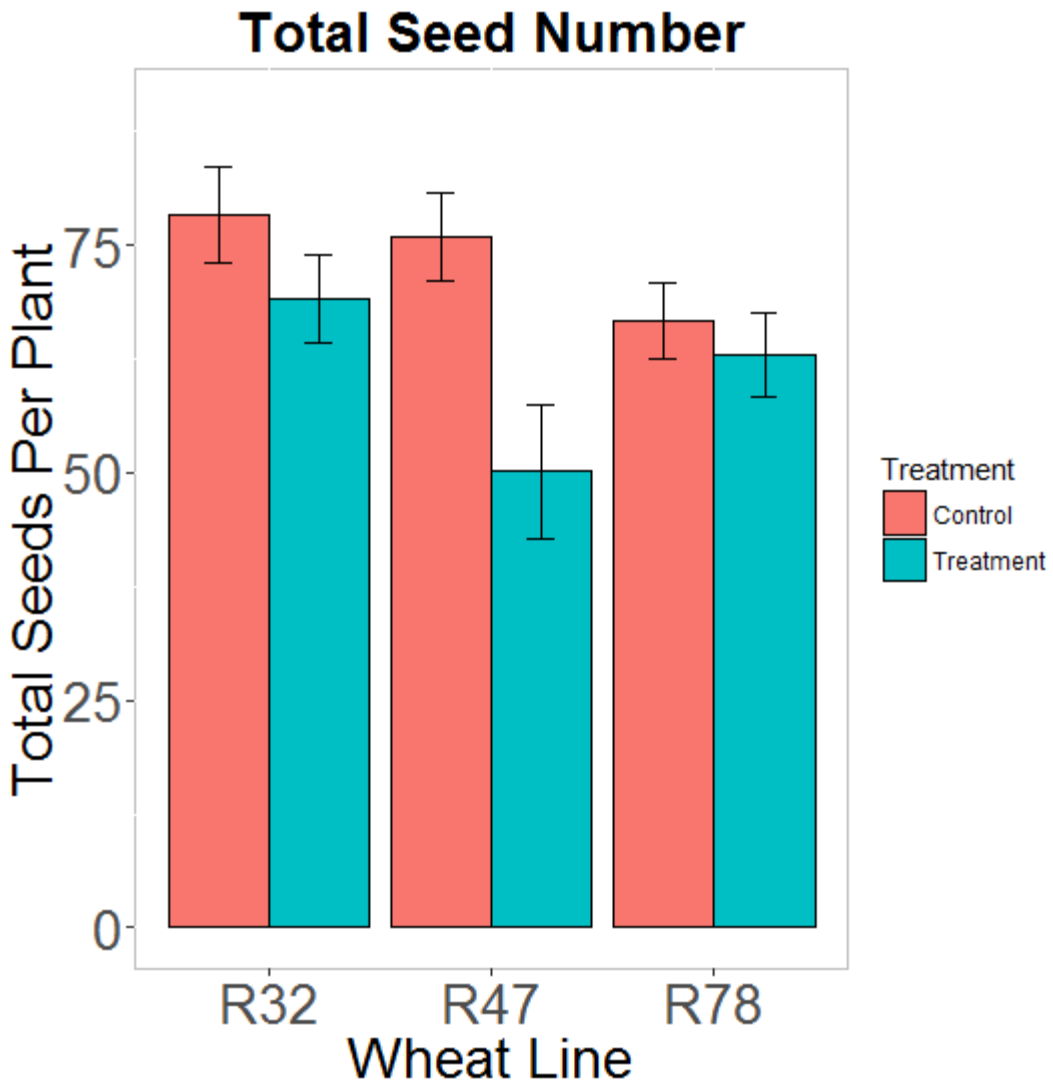

Figure 4.5 Relationship between total seed number per plant, wheat line, and treatment. Wheat lines: resistant 25R32 (R32), susceptible Pioneer variety 25R47 (R47), and tolerant Pioneer variety 25R78 (R78) when infested (blue) and uninfested (pink). Error bars represent standard errors of each sample.

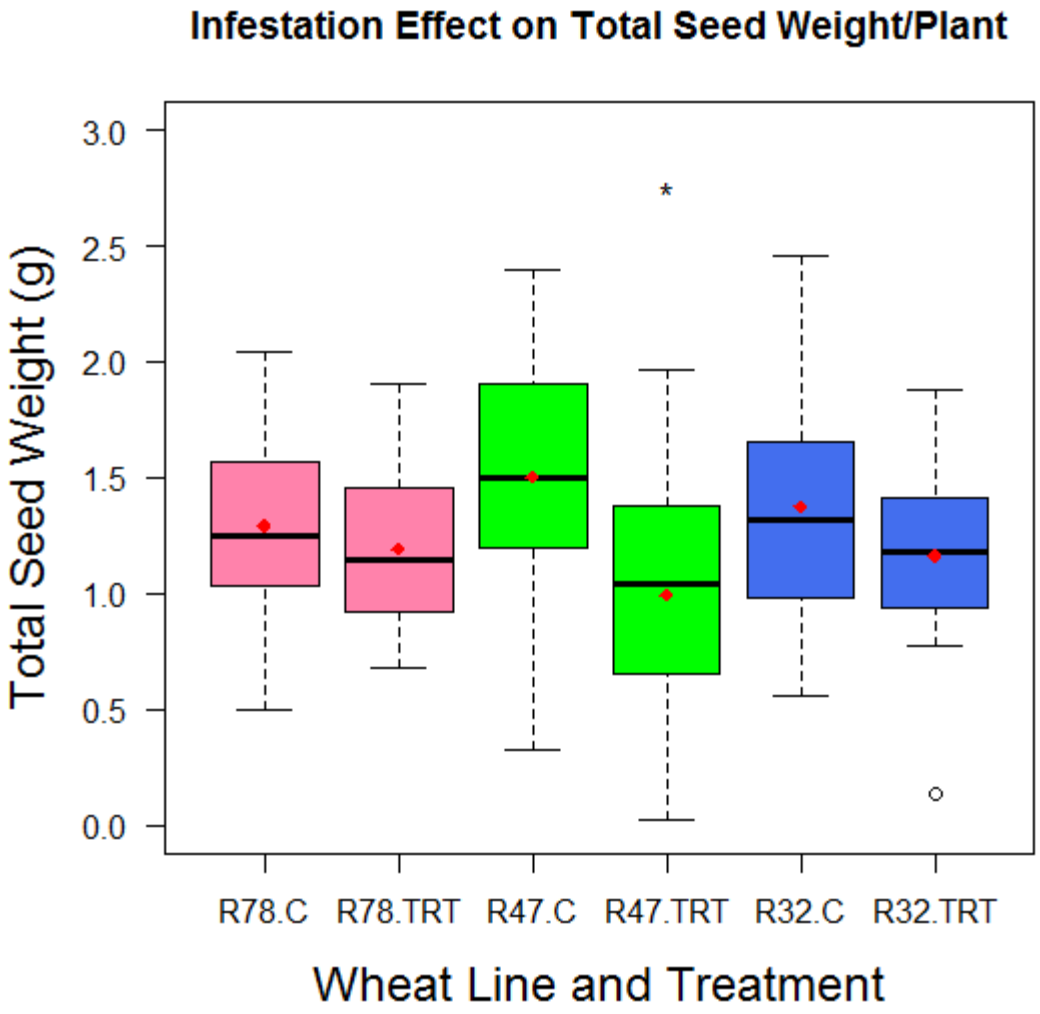

Figure 4.6 Relationship between the total seed weight per plant, wheat line, and treatment. Treatments where total seed weight per plant means differ significantly ($P < 0.05$) from their corresponding controls are indicated by a black asterisk. The box plot highlights the sample mean (red dot), median (thick dark line), first and third quartiles (lower and upper edges), and minimum and maximum (lower and upper range bars). Possible outliers in a sample are indicated by the open circles.

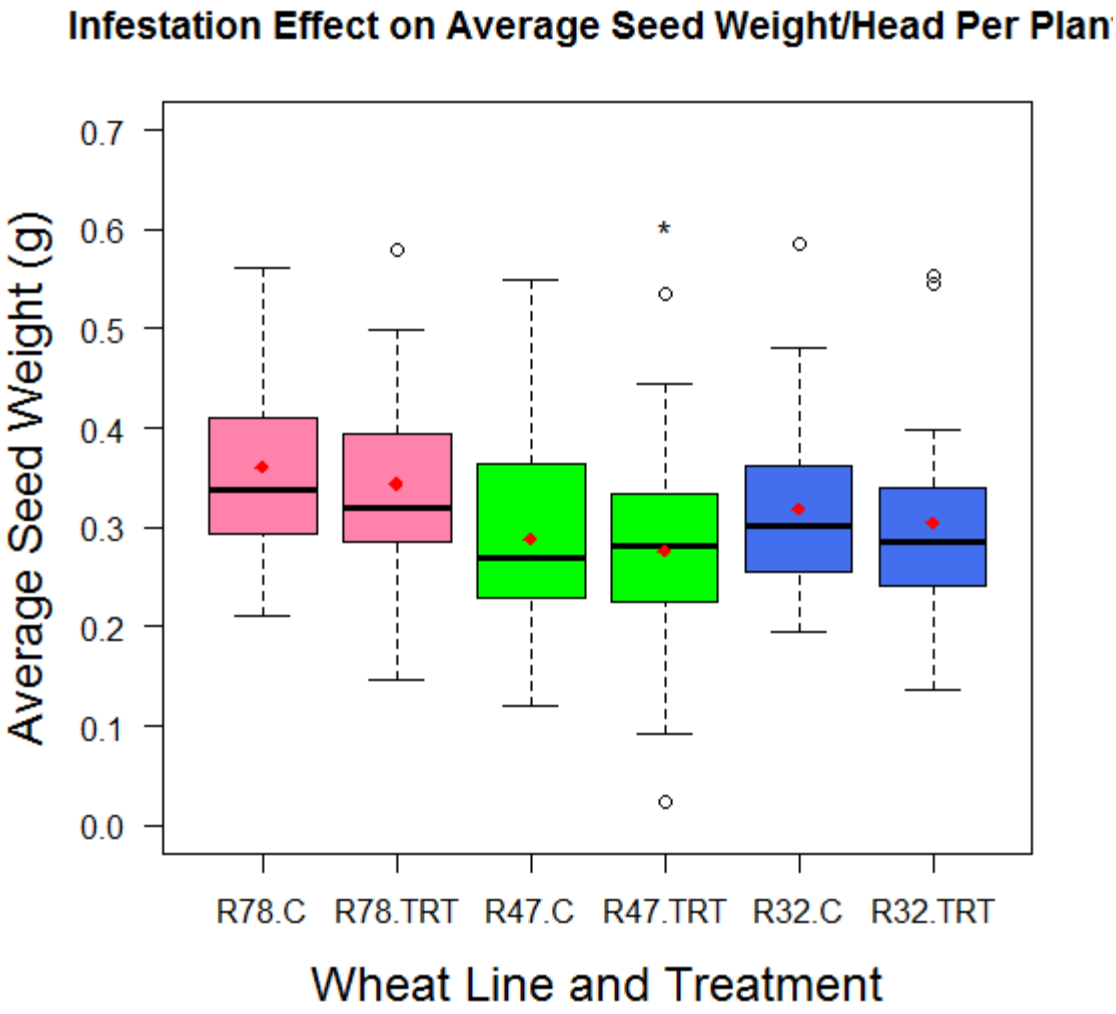

Figure 4.7 Relationship between the average seed weight per plant, wheat line, and treatment. Treatments where average seed weights per plant means differ significantly ($P < 0.05$) from their corresponding controls are indicated by a black asterisk. The box plot highlights the sample mean (red dot), median (thick dark line), first and third quartiles (lower and upper edges), and minimum and maximum (lower and upper range bars). Possible outliers in a sample are indicated by the open circles.

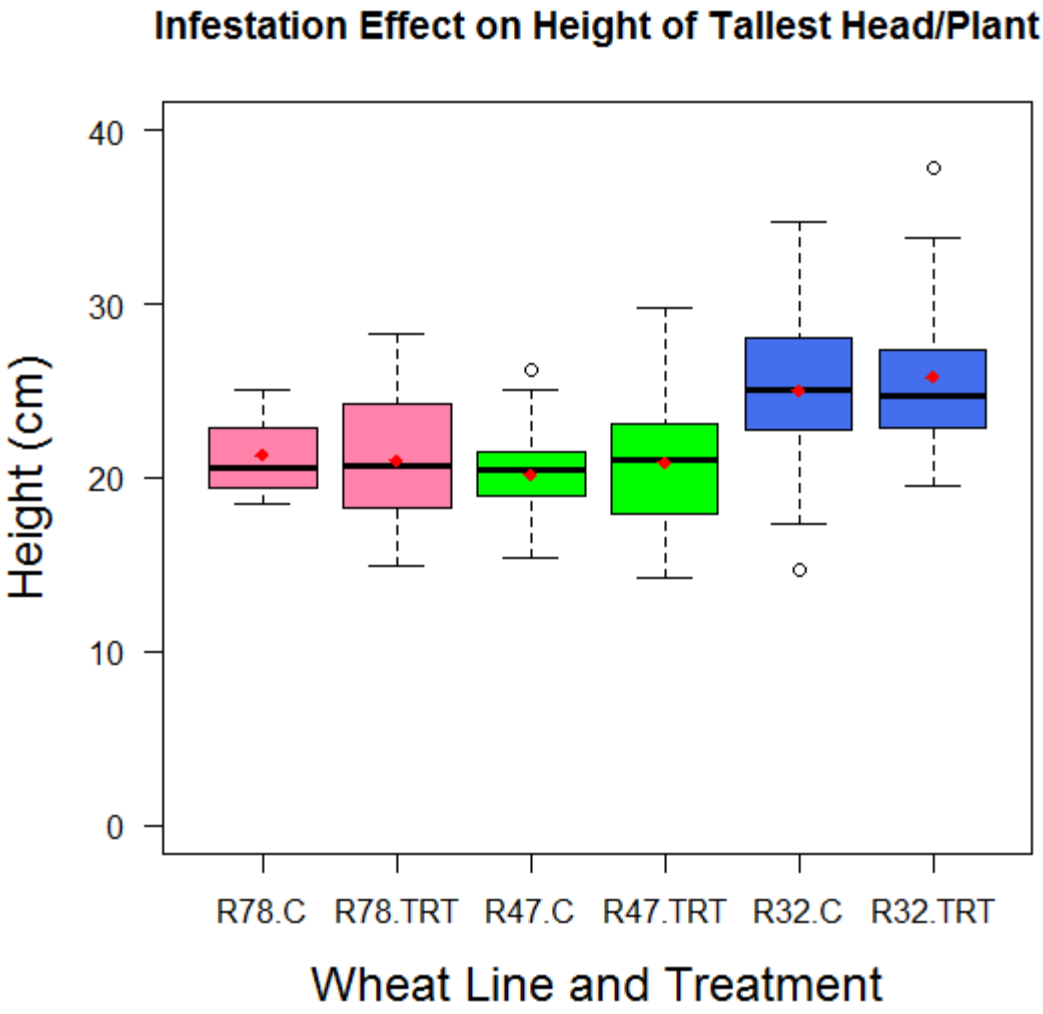

Figure 4.8 Relationship between the height of the tallest head/plant, wheat line, and treatment. Treatments where the tallest head height/plant means differ significantly ($P < 0.05$) from their corresponding controls are indicated by a black asterisk. The box plot highlights the sample mean (red dot), median (thick dark line), first and third quartiles (lower and upper edges), and minimum and maximum (lower and upper range bars). Possible outliers in a sample are indicated by the open circles.

CHAPTER 5. SUMMARY OF OBJECTIVES AND CONCLUSIONS

5.1 Review of Main Objectives

Hessian fly infestations can cause crop loss in wheat, affecting yields. Currently, resistance genes in wheat are the primary control method, but come at a cost due to the selection pressures they place on fly populations. Tolerance might be a useful tool to manage Hessian flies, with the potential for plants to grow even when infested without placing selection pressures on fly populations. Since there has been no novel research solely on tolerance in wheat to Hessian flies, the goal of this project was to examine and understand whether the putative tolerant Pioneer variety 25R78 truly demonstrated tolerance, whether this line would trigger larval death, and how tolerance would be demonstrated during infestation.

Consequently, the three goals of this study stated in Chapter 1 include:

1. Investigate the growth effects from Hessian fly infestation on putative tolerant Pioneer variety 25R78 in two time sets: 16 and 32 days post infestation (dpi).
2. Further investigate the mechanism of tolerance through improved study design focusing on:

A) Investigating the growth effects from Hessian fly infestation on putative tolerant Pioneer variety 25R78 at 20 dpi.

B) Investigating the effects of tolerant plants on Hessian fly larvae, including size, survival, and position on the plant.

3. Investigate the effects of Hessian fly infestation on the yield of tolerant plants.

5.2 Summary and Conclusions

This research was conducted to investigate Hessian fly infestation effects on putative tolerant wheat variety's growth and yield as well as the putative tolerant wheat's effect on Hessian fly larvae. Three studies were performed to analyze effects of Hessian fly infestation on tolerant plant (Pioneer variety 25R78) growth and yield and tolerant plant effects on Hessian fly larvae.

There was no loss in leaf or tiller number between infested or uninfested tolerant plants for the 16, 20, and 32-day sets in the first and second studies. In the third study, there was no loss in tiller number between infested and uninfested tolerant plants. These results indicate that Hessian fly infestation has no significant effects on leaf or tiller number for tolerant Pioneer variety 25R78. This could prevent loss in photosynthetic material and biomass. Also, no tiller reduction could lead to greater grain production since the number of tillers per plant is a primary contributor to grain yield.

Total leaf length of the tolerant line did not appear to be affected by infestation for the 32-day set of the first study as well as the 20-day set in the second study, but was

affected in the 16-day set of the first study. Although the 16 and 32-day sets are not directly comparable, it might demonstrate initial stunting of growth around 16 dpi with recovery in growth by 32 dpi. Even though total leaf lengths were significantly smaller at 16 dpi, the infested tolerant plants showed significantly greater total leaf lengths compared to the infested plants of the two susceptible lines. The 32-day set and the set from the second experiment demonstrated either no loss in leaf length or a recovery in growth.

Leaf growth rates (GR) showed significant effects in the first and second studies. In the 16-day set of the first study and the 20-day set in the second study, infested tolerant plants had significantly smaller leaf GR compared to the uninfested plants. Infested tolerant plants in the 32-day set of the first study showed no significant difference in GR. This might indicate potential stunting of the entire plant or in individual leaves, leading to effects in leaf GR. However, the infested resistant plants in the second study showed similar effects in leaf GR. Additionally, there was no significant difference between the leaf GR for infested resistant and infested tolerant plants, indicating growth effects on leaf GR was not exclusive to the tolerant plants. The absence of growth effects in leaf GR for the 32-day set possibly indicates recovery from or prevention of stunting of leaves. This might benefit the plant's survival and yield in the long-term.

The number of larvae were significantly fewer on tolerant plants than on Pioneer variety 25R75 at 16 dpi and both 'Newton' and Pioneer variety 25R75 at 32 dpi. There was no significant difference in larvae number between infested tolerant and the other lines at 20 dpi. The difference in larvae number in the 16- and 32- day sets might be the result of less observation since the 20-day set had continuous observation for larvae.

Since there were visible larvae on the susceptible and tolerant lines in the 20-dpi set, the larvae could fall from the plant into the soil in the first study, reducing larval number observed at the end of the study. Larval area was analyzed as a measurement of general antibiosis. The average larval area for tolerant plants was significantly smaller than larval area for ‘Newton’ at 16 dpi, both susceptible lines at 32 dpi, and both two susceptible lines at 20 dpi. There was no significant difference in average larval area between infested tolerant and ‘Iris’ lines at 20 dpi. However, no dead red larvae were found on tolerant plants.

Smaller larval size on tolerant plants could be attributed to antibiosis from lectins or other defense responses. However, given the survival of the larvae, fatal larval antibiosis due to incompatible interactions caused by resistance genes appears to be unlikely. The absence of larval death could prevent selection pressures from being placed on fly populations. Smaller larvae might also be caused by plant growth recovery. If the plant continues to grow without permanent stunting, the larvae could be displaced on the plant and away from feeding sites, reducing the time for feeding and reducing larval size. If the larvae are displaced after the first instar, the larvae cannot reposition themselves to make new feeding sites due to the lack of creeping pads.

In the second study, there were significantly more visible larvae on tolerant plants than the other three lines. Visible larvae were located significantly higher on tolerant plants than the other plants. They also were located significantly higher compared to the first ligule. In fact, only the tolerant plants showed visible larvae above the first ligule and leaf sheath. This might indicate larvae are pushed out of the leaf sheath. It is possible that as the plant grows, larvae are displaced from beneath the leaf sheath and away from

the feeding sites. This would affect feeding and development indirectly, thus explaining the smaller larvae. Also, the larvae would be exposed to adverse conditions such as desiccation, parasitism, and drowning. If the cause of the smaller larvae was the growth of the plant, this would indicate the absence of antibiosis due to the absence of a direct plant effect on larval growth. Instead, plant growth could indirectly affect larvae. The indirect effect on larval growth and survival, as well as the absence of a known *R* gene could reduce fly populations without placing selection pressures on the populations.

The third study demonstrated the absence of yield effects in tolerant plants from infestation. There were no significant effects on head or tiller number, total seed number/weight, average seed number/weight, tallest head height, tallest leaf height, or average head length. Tolerant plants showed significantly smaller tallest leaves and tallest heads, but no significant differences for the other yield measurements. Tolerant plants showed slightly great-er average seed weights/head compared to both susceptible and resistant plants, but not significantly more. Tolerant plants showed significantly greater average seed numbers per head than susceptible plants.

Overall, the putative tolerant plants appear to be able to prevent leaf or tiller loss despite infestation while reducing stunting in leaf growth rates and total leaf lengths. They also appear to be able to overcome stunting in individual leaf lengths with stunting in only the third and fourth leaves. Tolerant Pioneer variety 25R8 has the potential to be cultivated without the concerns of yield loss from larval feeding and new virulent biotype formation. This combines the benefit of the absence of selection pressures on fly populations present for susceptible lines with the benefit of successful plant growth and yield present in resistant lines. It can be concluded that Pioneer variety 25R78 is tolerant

to Hessian fly attack, tolerating damage caused by larval feeding through nutritive tissue and preventing damage in some cases such as reductions in leaf and tiller production. This wheat line successfully fits the definitions of tolerance from Section 1.4.1, including the ability to recover, grow, and reproduce. With no larval death placing selection pressures on the fly populations and the ability of the plants to produce a comparable yield to uninfested plants, it can be concluded that tolerance could be a useful tool to control Hessian flies.

APPENDICES

Appendix A Additional Data for Chapter 2

Larvae Number

At 16 dpi, Pioneer variety 25R78 had significantly fewer larvae than ‘Newton’ ($Z = -2.717$, $P = 0.018$). Pioneer variety 25R75 had a mean ± SE of 27.63 ± 6.956 larvae. Pioneer variety 25R78 had a mean ± SE of 15.53 ± 2.288 larvae. ‘Newton’ had a mean ± SE of 34.20 ± 3.640 larvae. The infested plants of the three lines showed a significant difference in number of larvae at 32 days. Both ‘Newton’ ($Z = 2.993$, $P = 0.008$) and Pioneer variety 25R78 ($Z = -5.578$, $P = < 0.001$) showed significantly fewer larvae than Pioneer variety 25R75. In the 32-day set, Pioneer variety 25R75 had a mean ± SE of 37.24 ± 4.940 larvae. Pioneer variety 25R78 had a mean ± SE of 6.500 ± 2.200 larvae. ‘Newton’ had a mean ± SE of 19.69 ± 2.916 larvae. No visible dead red larvae were observed on any of the infested plants.

Average Larval Area

For the 16 dpi set, the mean ± SE of larval area for Pioneer variety 25R75 was 1.991×10^{-5} µm ± 1.492×10^{-5} µm. The mean ± SE of larval area for Pioneer variety

25R78 was $1.033 \times 10^{-6} \pm 1.184 \times 10^{-5}$ µm. The mean ± SE of larval area for ‘Newton’ was $1.091 \times 10^{-6} \pm 7.567 \times 10^{-4}$ µm. The average larval area of infested tolerant plants was significantly smaller than that of ‘Newton’ (P = 0.018), but not Pioneer variety 25R75 (P = 0.190) (Table 2.1; Fig. 2.1a). For 32 dpi, the mean ± SE of larval area for Pioneer variety 25R75 was $1.991 \times 10^{-6} \pm 1.030 \times 10^{-5}$ µm. The mean ± SE of larval area for Pioneer variety 25R78 was $1.606 \times 10^{-6} \pm 1.655 \times 10^{-5}$ µm. The mean ± SE of larval area for ‘Newton’ was $2.102 \times 10^{-6} \pm 2.158 \times 10^{-5}$ µm. The average larval area of infested tolerant plants was significantly smaller than that of ‘Newton’ and Pioneer variety 25R75 (P = 0.040 and P = 0.006, respectively) (Table 2.1, Fig. 2.1b).

Average Leaf Growth Rate

At 16 dpi, the infested tolerant plants had significantly smaller leaf GR than uninfested tolerant plants (P = < 0.001) (Table 2.4; Fig. 2.4a). Infested susceptible ‘Newton’ and Pioneer variety 25R75 (P = < 0.001 for both varieties) showed significantly smaller leaf GR than uninfested plants. Infested tolerant plants had significantly greater leaf growth rate compared to infested ‘Newton’ plants, but not infested Pioneer variety 25R75 plants (P = < 0.001 and P = 0.576, respectively). At 32 dpi, there was no significant difference between leaf GR of infested and uninfested tolerant plants (P = 0.082) (Table 2.4; Fig. 2.4b). Both infested ‘Newton’ and Pioneer variety 25R75 (P = < 0.001 for both varieties) showed significantly smaller leaf GR compared to uninfested plants. Infested tolerant plants had significantly greater leaf growth rate than infested plants of ‘Newton’ and Pioneer variety 25R75 (P = < 0.001 for both varieties) (Fig. 2.4b).

Appendix B Additional Data for Chapter 3

Larvae Number

Wheat line had a significant effect on the number of larvae (F = 3.181, df = 2, P = 0.036) (Table 3.4). The sum of squares was 719.1 and the mean of squares was 239.7. However, there was no significant difference in the number of larvae between the tolerant line and susceptible variety 25R75, susceptible variety 'Newton', and resistant variety 'Iris' (P = 0.211, P = 0.440, and P = 0.903, respectively) (Table 3.5). Pioneer variety 25R75 had a mean ± SE of 16.07 ± 2.447 larvae. Pioneer variety 25R78 had a mean ± SE of 8.500 ± 2.299 larvae. 'Newton' had a mean ± SE of 14.89 ± 3.289 larvae. Resistant variety 'Iris' had a mean ± SE of 5.429 ± 2.698 larvae. No visible dead red larvae were found observed on any of the infested plants.

Average Larval Area

Larval size differed significantly by wheat line (F = 42.99, P = < 0.001). The sum of squares was 3.179×10^{13} and the mean of squares was 1.060×10^{13}. Infested

tolerant Pioneer variety 25R78 showed significantly smaller larvae than Pioneer variety 25R75 or ‘Newton’ (P = < 0.001 for both varieties) (Table 3.5). There was no significant difference in larval size between Pioneer variety 25R78 and resistant ‘Iris’ (P = 0.988) (Table 3.5; Fig. 3.1).

Leaf Growth Rate

The treatment (F-value = 145.8, P = < 0.001), line (F-value = 7.825, P = < 0.001), and the interaction between the two (F-value = 6.065, P = < 0.001) appeared to have a significant impact on leaf growth rate (Table 3.2). The infested tolerant plants showed significantly smaller leaf growth rate compared to uninfested tolerant plants (P = 0.002) (Table 3.3; Fig. 3.6). Infested ‘Iris’, ‘Newton’, and Pioneer variety 25R75 also showed significantly smaller growth rate compared to their uninfested counterparts (P = 0.048, P = < 0.001, and P = < 0.001, respectively). There was no significant difference in leaf growth rate between the infested tolerant and infested ‘Iris’ plants, as well as infested ‘Newton’ and Pioneer variety 25R75 (P = 0.999, P = 0.054, and P = 0.596, respectively).

Number of Visible Larvae above the First Ligule

Line had a significant impact on the number of visible larvae found above the first ligule (F-value = 3.926, df = 3,93, P = 0.011). The tolerant plants had significantly greater visible larvae above the first ligule than ‘Newton’, “Iris’, and Pioneer variety 25R75 (P = 0.048, P = 0.037, and P = 0.018, respectively). The mean (+-SE) number of visible larvae above the 1st ligule for tolerant Pioneer variety 25R78 was 3.14 larvae. The means for the other lines were identical with a mean of 0.

Appendix C Additional Data for Chapter 4

Tallest Head Height

The line (F-value = 25.016, df = 2, P = < 0.001), but not treatment (F-value = 0.680, df = 1, P = 0.411) or interaction of the two (F-value = 0.285, df = 2, P = 0.752) appeared to have a significant impact on the height of the tallest head per plant (Table 4.3). There appeared to be no significant difference in tallest head height between infested and uninfested tolerant plants (P = 0.999) (Fig. 4.8). The same is true for Pioneer 25R32 and Pioneer variety 25R47 (P = 0.981 and P = 0.994, respectively). There was no significant difference in tallest head height between infested tolerant plants and infested Pioneer variety 25R47 plants (P = 0.999). Infested tolerant plants showed significantly shorter head heights than infested Pioneer variety 25R32 plants (P = < 0.001). Even uninfested tolerant plants showed significantly shorter tallest head heights than uninfested Pioneer variety 25R32 plants (P = 0.007) (Table 4.4).

Average Head Height

The line (F-value = 28.54, df = 2, P = < 0.001), but not treatment (F-value = 0.907, df = 1, P = 0.342) or interaction of the two (F-value = 0.148, df = 2, P = 0.863) appeared to have a significant impact on the average head height per plant (Table 4.3). There appeared to be no significant difference in average head height between infested and uninfested tolerant plants (P = 0.999). The same is true for Pioneer 25R32 and Pioneer variety 25R47 (P = 0.948 and P = 0.997, respectively). There was no significant difference in average head height between infested tolerant plants and infested Pioneer

variety 25R47 plants (P = 0.990). Infested tolerant plants showed significantly shorter average head heights than infested Pioneer variety 25R32 plants (P = < 0.001). Even uninfested tolerant plants showed significantly shorter average head heights than uninfested Pioneer variety 25R32 plants (P = < 0.001) (Table 4.4).

Tallest Leaf Height

The line (F-value = 10.99, df = 2, P = < 0.001), but not treatment (F-value = 0.113, df = 1, P = 0.737) or interaction of the two (F-value = 0.275, df = 2, P = 0.760) appeared to have a significant impact on the height of the tallest leaf per plant (Table 4.3). There appeared to be no significant difference in tallest leaf height between infested and uninfested tolerant plants (P = 0.999). The same is true for Pioneer 25R32 and Pioneer variety 25R47 (P = 1.000 and P = 0.948, respectively). There was no significant difference in the height of the tallest leaf between infested tolerant plants and infested Pioneer variety 25R47 plants (P = 0.401). Infested tolerant plants showed significantly shorter leaf heights than infested Pioneer variety 25R32 plants (P = 0.018). Even uninfested tolerant plants showed significantly shorter tallest leaf heights than uninfested Pioneer variety 25R32 plants (P = 0.012) (Table 4.4).

Plant Survival

There is no evidence that plant survival depends on wheat line and infestation (χ^2 = 7, df = 5, P = 0.221). The only wheat line that showed plant death was susceptible Pioneer variety 25R47 when infested. Ten infested susceptible plants died while no other

plants died. However, this was not significantly different from the other lines and treatment.

Appendix D Plant Cover Picture

Figure D.1 Plant Covers. Left: Plant infestation cover for Chapter 2. Middle: Plant infestation cover for Chapters 3 & 4. Right: Fly emergence cover for Chapter 4.